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THE STATUS AND AFFINITIES OF THE ANTHOMEDUSAN *PARAGOTOEA BATHYBIA* KRAMP, 1942

BY

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[Accepted 11th November 1958]

(With 1 figure in the text)

CONTENTS

	Page
Introduction	171
Paragotoea from the Western Approaches	172
(a) Specimen A	172
(b) Specimen B	172
(c) Specimen C	174
The type specimen of *Paragotoea bathybia*	174
The status of *Paragotoea*	175
The Paragotoeidae Fam. nov.	176
Acknowledgments	176
Summary	177
References	177

INTRODUCTION

During an examination of the catches made by the Marine Biological Association's research vessel " Sarsia " in the approaches to the English Channel and off the edge of the Continental Shelf, three rather unusual anthomedusae were found. Two of these were collected in 1956 and the other in 1957. These proved to be *Paragotoea bathybia* Kramp (1942), a rare species known only from a single specimen taken off Frederikshaab, Greenland.

Paragotoea bathybia as described by Kramp has an " umbrella 1·3 mm. high and 1·6 mm. wide, with flattened top and thin walls. Manubrium short and thick, with bulging sides and a simple mouth opening. Four radial canals, narrow at their origin, considerably widened in their distal parts; circular canal narrow. Four marginal bulbs of very unequal size with an ectodermal spur clasping round the umbrella margin. One stiff and solid tentacle with a large terminal knob of nematocysts. Numerous nematocysts scattered over the exumbrella ".

On the basis of these characteristics Kramp placed *P. bathybia* in the family Tubulariidae. These new specimens however show that the stomach changes shape becoming quadrate in the apical region and extends laterally as four pouches, and that the tentacles increase from one to four, as the medusa grows from juvenile to adult. Four marginal tentacles are known for many species of the Tubulariidae but a quadrate stomach and four stomach pouches are unknown in the family and raise doubts that *P. bathybia* is a tubularid.

* Nuffield Fellow, 1958. British Museum (Natural History).

PARAGOTOEA FROM THE WESTERN APPROACHES

(a) Specimen A

Locality : 6/5/57, 47° 50′ N, 9° 15′ W, 1000 fath. wire, 0155–0235 hr. (Fig. 1, A).

This is the largest known specimen of *P. bathybia*. The bell is rather contracted, 3·85 mm. in height and 3·99 mm. in greatest diameter ; the type specimen is about one-third this size. Only the stumps of the four tentacles remain, but these appear to have the characteristic delicate, thin, ectoderm, and very thick mesogloea, and prominent layer of endodermal cells described by Kramp for the species. A good deal of the exumbrellar epithelium is missing as well as most of the nematocysts on the four exumbrellar pads at the bases of the radial canals, but the area occupied by the pad is outlined clearly as bluntly triangular in shape with the apex of the triangle towards the top of the bell, and the base curves under the margin of the bell. (Fig. 1, C & D). The vertical height of the nematocyst pads is approximately 1·05 mm. and their basal length is 0·35 mm.

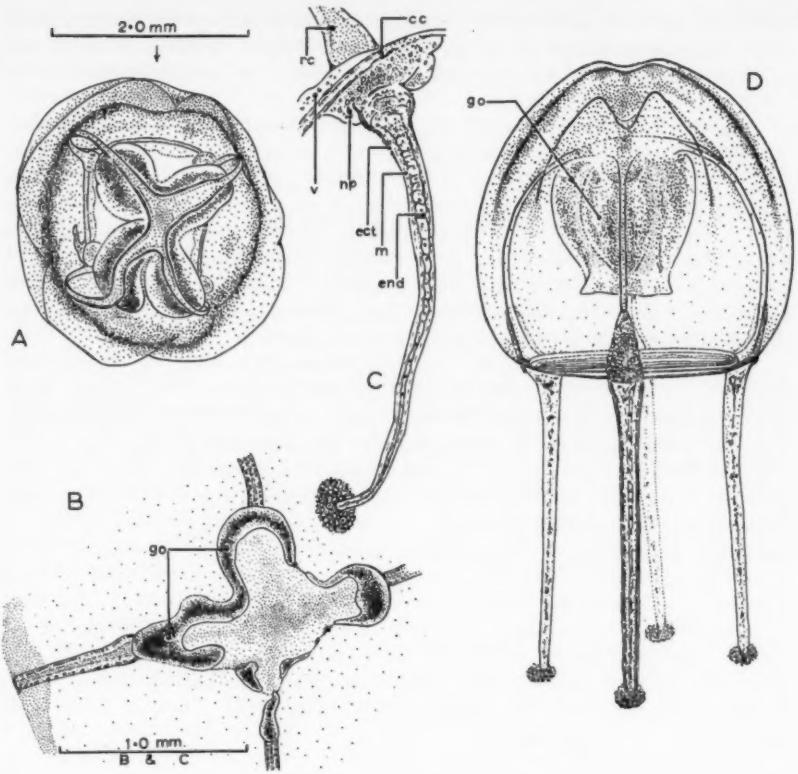
The subumbrellar cavity is well defined with four interradial apical projections (Fig. 1, D). Possibly the stomach has a small peduncle and these projections may then be due to a downward contraction of the apical region of the stomach when the medusa was preserved. There are however smaller, but similar, projections in the better preserved younger specimens. Each projection in this large specimen is approximately 0·70 mm. in vertical height and 0·56 mm. in diameter at the base and is thus a prominent feature of the apical region of the subumbrellar cavity. Although the apical bell region is contracted it appears that the form of the apex of the bell is more quadrate than dome-shaped, with possibly four conical projections corresponding in position to those in the subumbrellar cavity. The mouth of the subumbrellar cavity is rectangular in shape with bluntly rounded corners, and the longer side is 1·48 mm. in length and the shorter 1·05 mm. Excluding the apical projections the subumbrellar cavity is 2·45 mm. in height and 3·36 mm. in width.

The mesogloea of the bell averages 0·35 mm. in thickness but is thickest at the apex where it is difficult to obtain an accurate measurement because of the contraction of the bell. The length of the stomach and the manubrium combined, that is, measured from mouth to apex, is 1·75 mm. and the diameter of the stomach at the apex is 2·10 mm. The width across the manubrium at its mouth is approximately 0·70 mm. The gonad extends over the gastric pouches, down onto the manubrium and measures 1·05 mm. in length from the apex of the stomach to its termination on the manubrium. The four radial canals are approximately 0·07 mm. in diameter, except where they conspicuously increase in width a little before their entry into the ring canal. In this specimen, it is not possible to give an accurate measurement of the broad basal region of the canals. The ring canal is rather narrow and approximately 0·07 mm. in diameter.

(b). Specimen B

Locality : 4/7/56, Haul 9, Bay of Biscay, 47° 02′ N, 5° 50′ W, 790–980 fath., 2145–2306 hr. (Fig. 1, B and C).

This specimen is approximately half the size, and not as contracted as the



A, Medusa, specimen " A ", viewed from the apex to show the quadrate stomach and four stomach pouches.

B, Medusa, specimen " B ", looking upwards into the subumbrellar cavity to show the remaining portion of gonad encircling the stomach (stomach and manubrium damaged and not shown).

C, Bell margin and tentacle of specimen " B " (bell turned inside out).

D, Diagram of adult medusa to show the probable form of the bell and marginal tentacles when extended.

cc, ring canal ; ect, ectoderm ; end, endoderm ; go, gonad ; m, mesogloea ; np, nematocyst pad ; rc, perradial canal ; v, velum.

large specimen described above. The bell is 2·0 mm. in height and 2·10 mm. in greatest width. One complete tentacle and the stump of a second in the opposite perradial position can be seen. The complete tentacle is broad at the base, gradually tapering to a very narrow tip which terminates in a large disc-shaped cluster of nematocysts. The total length of the tentacle including the terminal nematocysts disc is 2·4 mm., i.e. slightly greater in length than the height of the bell. The nematocyst cluster of stenoteles and atrichs is 0·07 mm. in thickness and 0·21 mm. in diameter. The exumbrellar nematocyst pads

overlying the base of the radial canals show clearly, and approximate in shape to an equilateral triangle with sides 0·06 mm. in length. There is no significant difference in the size of the pads and the nematocysts are so closely packed that the radial canal cannot be seen underneath.

The four radial canals have a diameter of 0·08 mm. for two-thirds of their length from the stomach to the margin, but, as in the large specimen, they widen out markedly in the region below the nematocyst pads before joining the ring canal. This wide region of the radial canal is similar in shape to that of the nematocyst pad but slightly smaller in area. Its entry into the ring canal is approximately 0·50 mm. in width. The manubrium and stomach pouches are damaged but the apical stomach is characteristically quadrate in shape and small portions of the encircling gonad can be seen. The diameter of the stomach viewed from the apex is 1·0 mm.

(c). Specimen C.

Locality : 7/2/56, Haul 1, A. Bay of Biscay, 46° 45′ N, 5° 43′ W, 477 fath. wire 1750–2037 hr.

This third specimen is approximately the same size as specimen " B " above with a bell 2·0 mm. by 2·0 mm. Where accurate measurements were possible these also were essentially the same as in specimen " B ". The stomach and manubrium are in poor condition, but small gastric pouches and an incipient gonad can be recognized. The apical region of the stomach is not so obviously quadrate as in the other " Sarsia " specimens. A single tentacle is present and is similar in structure and form to that seen in the specimen described above and is approximately equal in length to the height of the bell. I am indebted to Dr Russell for colour notes on this specimen. The marginal nematocyst pads were a bright orange-yellow and the apical region of the stomach a paler, but similar colour to the pads. The rest of the body and tentacle were transluscent.

THE TYPE SPECIMEN OF *PARAGOTOEA BATHYBIA*

Interpretation of the structures present in the type specimen of *P. bathybia* is now much easier because of the knowledge gained from the " Sarsia " specimens. The type specimen appears to have become distorted during fixation and preservation and is somewhat damaged. This seems to have led to some misinterpretation of the size of the exumbrellar nematocyst pads, and the shape of the bell.

The type specimen is the smallest of those known for the species and is a juvenile. The bell is only 1·3 mm. in height and 1·60 mm. in width. The radial canals are 0·60 mm. in diameter for two-thirds of their length, and then widen out as described above towards the margin of the bell. No accurate measurement of this latter region is possible. A single tentacle 1·30 mm. in length is present and its disc-shaped terminal nematocyst cluster of stenoteles and atrichs is 1·30 mm. in diameter and 0·15 mm. in thickness.

The exumbrellar nematocyst pads appear to be damaged and it seems likely on the evidence of the " Sarsia " specimens that they are of approximately equal size and not as formerly descibed—one large pad overlying the base of the radial canal in the radius bearing the single tentacle, and the other three

becoming progressively smaller in a clockwise direction. Moreover, the asymmetry of the bell figured for this specimen seems to be due to a markedly unequal contraction due to fixation and preservation and is therefore in all probability not the natural shape of the bell. Although damaged, there are present small, but recognizable stomach pouches and patches of gonad. The apical region of the stomach is not obviously quadrate and is rather similar to that shown by " Sarsia " specimen " C ".

THE STATUS OF *PARAGOTOEA*

On the evidence, *Paragotoea bathybia* as described by Kramp is a young medusa. Small differences between Kramp's specimen and mine can be shown to be due to damage and a difference in age. Medusae with a bell height of up to approximately 2·0 mm. seem to have a single tentacle, four small gastric pouches, a four-lobed but not distinctly quadrate, apical region to the stomach and have incipient patches of gonad on the stomach. The pads of exumbrellar nematocysts are very prominent in these young animals. As the medusa grows, the tentacles increase in number to four, the apex of the stomach becomes clearly quadrate, four lateral stomach pouches are formed and the gonad surrounds the stomach and extends over the stomach pouches and part of the manubrium, and the exumbrellar nematocyst pads (while remaining approximately triangular in shape) grow unequally, and the vertical height lengthens more rapidly than that of the base and the pads are not as prominent a feature of the exumbrellar as they are in a younger medusa.

The characteristics of the family Tubulariidae have been noted above. *P. bathybia* as described by Kramp (1942) was readily recognizable as a member of the Tubulariidae, but the knowledge gained from the " Sarsia " specimens shows that it can no longer be retained within this family as all tubularid medusae have a tubular, codonid, non-quadrate stomach. Adult *Paragotoea* have a quadrate stomach and four large stomach pouches, with the gonad extending over the stomach pouches and part of the manubrium. Only one group of the Capitata have a quadrate stomach and stomach pouches namely, members of the Superfamily Moerisioidea Rees, 1958, containing one family, the Moerisiidae Poche, 1914. Species of the family Moerisiidae have a cnidome of stenoteles, atrichs and desmonemes, that is, a cnidome essentially similar to that of *Paragotoea* (stenoteles and atrichs). Therefore *Paragotoea* has two characteristics in common with the Moerisiidae. However in the Moerisiidae the stomach pouches are much larger and extend at least half-way down the radial canals, whereas the stomach pouches of *Paragotoea*, extend barely, if at all, down the radial canal. Moreover in the Moerisiidae, interradial tentacles are usually present between the four perradial tentacles.

Paragotoea seems to be intermediate between the Tubulariidae and Moerisiidae. When young it resembles a tubularid as it has exumbrellar nematocyst pads that curl under the margin of the bell (also present in the adult) and a tubular codonid stomach with a circular apical region. When adult, *Paragotoea* exhibits a simple kind of quadrate stomach and stomach pouches which reach their full development in the Moerisiidae. However, there are no ocelli and no extra marginal tentacles, features that are characteristic of the Moerisiidae.

THE PARAGOTOEIDAE fam. nov.

All in all, *Paragotoea* seems most nearly related to the Tubulariidae but for the reasons stated above cannot be placed in this family. It seems therefore best to create a new family the Paragotoeidae, which in evolution seems to have diverged from the main stock which gave rise to the Tubulariidae, but to have arisen late in the evolution of the Superfamily. The medusa alone is insufficient to determine the relation of this new family to the Superfamilies Tubularoidea and Moerisioidea. Although the characters of the medusae suggest the retention of this family in the Tubularoidea rather than its transfer to the Moerisioidea its systematic position cannot be regarded as secure until its hydroid is known. The hydroids of the Tubularoidea possess two sets of tentacles, capitate, filiform or moniliform in type whereas the Moerisioidea have a single whorl of capitate or moniliform tentacles.

The family Paragotoeidae is defined as follows :

Family Paragotoeidae n. fam.

Bell with or without apical projections ; with numerous, scattered exumbrellar nematocysts and with four exumbrellar nematocyst pads overlying the base of the radial canals. There are four stiff marginal tentacles each with a broad base, tapering gradually to terminate in a disc-shaped nematocyst cluster. There is a bicnidome of stenoteles and atrichs. The manubrium has a simple mouth and does not extend beyond the umbrellar margin ; it has a round apical region in the young medusa but is quadrate in this region in the adult. The latter has four well developed lateral stomach pouches. The stomach pouches extend barely, if at all, down the radial canals. Four narrow perradial canals are present, these widen out for the basal third of their length and enter the ring canal by a broad opening. The gonad completely encircles the quadrate stomach and extends over the stomach pouches and partly on to the manubrium. Hydroid—unknown.

The material has been distributed as follows :—Specimen " A "—British Museum (Natural History), Reg. No. 1958.10.28.1 : Specimen " B "—Zoology Department, Victoria University of Wellington, New Zealand ; Specimen " C " —Plymouth Marine Biological Laboratory.

The position of *Gotoea* Uchida, 1927 remains to be assessed. It was regarded by Kramp (1942) as very closely allied, and possibly identical with *Paragotoea*, but the gonads of the former are " in the form of sausage-like processes in the interradii " and the central endodermal core of the tentacle in *Gotoea* is said to have more than one layer of endodermal cells, while that of *Paragotoea* has a single layer. Moreover, Uchida does not describe a quadrate apical region to the stomach for *Gotoea*. The known specimens of this genus are sufficiently large (bell 3·3 mm. in height) to show a quadrate apical region to the stomach if *Gotoea* is to be considered congeneric with *Paragotoea*. Therefore, as at present known, *Gotoea* Uchida 1927, is distinct from *Paragotoea* Kramp 1942, and is recognized here as a member of the family Tubulariidae.

ACKNOWLEDGMENTS

The author wishes to thank Dr W. J. Rees of the British Museum (Natural History) and Dr F. S. Russell for very helpful discussion ; and Dr F. Jensenius

Madsen, of the Universitetets Zoologiske Museum, Copenhagen, for allowing her to see the type specimen of *Paragotoea bathybia* Kramp.

SUMMARY

Three specimens of a rare anthomedusa collected by the R.V. "Sarsia" west of the English Channel, are described and compared with *Paragotoea bathybia* Kramp, 1942 and have been identified with it.

With age, the number of tentacles increases from one to four and a quadrate stomach with four stomach pouches is developed as the medusa matures.

A quadrate stomach is regarded as significant in determining the affinities of *Paragotoea* and a new anthomedusan family Paragotoeidae is created for it.

Pending the discovery of more specimens of the Japanese form, *Gotoea* Uchida 1927, is recognized as distinct from *Paragotoea* and retained in the family Tubulariidae.

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DEFAECATION IN THE FLYING LEMUR *CYNOCEPHALUS VARIEGATUS*

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[Accepted 11th November 1958]

(With 1 plate)

Animals which normally rest suspended in head-down posture are confronted with the special problem of avoiding fur-soiling during defaecation. Observations made separately on both wild and captive examples of the Malayan Flying-Fox (*Pteropus vampyrus*) show that this animal, which spends most of its time suspended head-downwards from its hind feet, has to reverse this position during defaecation and assume a head-upward position by the employment of the first digit of the forelimb.

Another animal experiencing this necessity for upright body-posture during defaecation is the Flying Lemur (*Cynocephalus variegatus*), restricted to Malaysia and the Phillipines. It feeds exclusively, as examination of its stomach contents shows, upon leaves. It spends most of its time either hanging head downwards, with feet clasped around a small branch (Plate, fig. a) or flattened against a tree trunk head-upwards (Plate, fig. b). Supported by its outspread patagia, it can and does glide for considerable distances from high trees, much in the manner of the flying squirrels in association with which it occurs. *Cynocephalus* differs however from the flying squirrel in that its patagium not only extends between the limbs but also connects the hind limbs to the entire length of the tail. The tail is normally carried folded under the abdomen, thus forming, with the patagium, a ' pocket ' over the hinder end of the belly (as evident in the Plate, fig. a). The anus being situate at the bottom of this ' pocket ' the difficulty imposed by defaecation is obvious.

Its restricted diet prevents the Flying Lemur from being successfully kept for long in captivity. Nevertheless, satisfactory personal observations have been made on defaecation in captive animals and photographs such as Plate, fig. c, have been obtained by way of record. From this photograph it will be noted that the animal has adopted the head-upwards body posture as in fig. b, but that the tail, instead of being drawn up under the abdomen, has been reverted so as to lie along the back, and that the ' pocket ' formed by belly wall, interfemoral patagium and tail has become everted with consequent exposure of the anus at the most dependent point of the trunk. With the adoption of this particular posture fairly copious faeces can be voided without risk of fur-soiling.

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A curious feature of the pelage is the bright orange colour of the fur within the ' pocket ', in contrast to the dark colour (resembling lichen-covered bark) of the fur on the dorsal body surface and the patagium. This orange patch remains hidden whilst the animal is at rest (Plate, figs. a & b), but is prominently displayed during defaecation. The tail tip is then held in normal fashion (Plate, fig. c) and exhibits its dark-coloured dorsal surface, while the orange-coloured ventral surface of the tail-membrane is clearly displayed.

No explanation can be offered for the curious colour disparity of the pelage. The pinnae also are bright red in colour and are always conspicuously displayed, so aiding recognition of the animal in the field.

EXPLANATION OF PLATE

Fig. a.—The normal resting position, hanging from a branch.

„ b.—The alternative resting position, spread-eagled against a trunk, which is usually larger than the trunk illustrated.

„ c.—Position of defaecation.

a

b

c

Defaecation in the Flying Lemur.

THE EFFECTS OF LOW TEMPERATURES ON THE PHYSIOLOGY AND DISTRIBUTION OF THE COD, *GADUS MORHUA* L., IN THE BARENTS SEA

BY

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[Communicated by Mr N. B. Marshall—Accepted 9th December 1958]

(With 1 plate and 4 figures in the text)

CONTENTS

	Page
Introduction	181
The hypothesis	182
Material and methods	183
Blood analyses	183
Histological techniques	184
Results of plasma analyses	185
Gill cytology	188
Chloride secretory cells	188
Mucous cells	191
Seasonal changes in the effects of low temperatures	191
General discussion and conclusions	193
Summary	197
Acknowledgments	198
References	198

INTRODUCTION

Over large areas the ocean provides a constant physical and chemical environment for its contained fauna, so that, in regions where marked hydrographical discontinuities or boundaries occur, changes in biological distribution might also be expected. The Svalbard shelf* in the Barents Sea is such a boundary region, where the warm waters of the West Spitzbergen Current, a continuation of the Gulf Stream system, meet cold waters of Polar origin which form the Bear Island Current. On this shelf it is common to find large numbers of cod (*Gadus morhua* L.) apparently localized along the boundary between the two water masses. At times the relationship between the distribution of the cod and the temperature on the sea bed may appear to be very close ; the concentration of the fish often seems to increase as the temperature gradient becomes steeper, and occasionally large numbers of cod are found in pockets of warm water almost surrounded by colder water (Lee 1952, Graham, *et al.* 1954). Commercial deep sea fishing vessels take advantage of these concentrations and may make catches as high as fifteen tons of cod per hour's fishing. Lee (1956) has summarised these observations, stating that paying catches of

* It is convenient to adopt the name " Svalbard " which the Norwegians use for Spitzbergen and its neighbouring islands, including Bear Island and Hope Island.

cod (i.e. more than 1½ tons per hour's fishing) are not taken below a temperature of 2°C., except during the summer months of July to September when the fish may enter cold Arctic water to feed heavily on planktonic krill and on the capelin (*Mallotus villosus*). Maslov (1944) and Saetersdal (1956) also report that in the central and eastern Barents Sea the limits to the distribution of the cod may occasionally be described in some detail by sea-bottom isotherms.

Although these observations on the distribution of the cod suggested a close relationship to temperature, emphasising the apparently limiting effect of temperatures below the critical 2°C., they did not necessarily imply active avoidance by the fish, because if the cod were associated with currents rather than temperatures, those cod living in the West Spitzbergen Current would only be found in warm water (i.e. above 2°C.) and would therefore appear to avoid the cold Arctic water; however, in this case, it would seem more difficult to account for the concentration of fish at hydrographic boundaries. The question therefore arose whether low temperatures did exert any direct effect upon the behaviour or physiology of the cod. In studying this problem, a hypothesis was first developed to explain the observed distribution of the cod in terms of the effects of low temperatures upon the fish. This hypothesis was then put to the test in the Barents Sea. The paper will present this study in chronological order, first developing the hypothesis, then giving the results, and finally discussing their significance.

THE HYPOTHESIS

Consideration of laboratory studies on the behaviour of fish in temperature gradients suggested that fish tend to select temperatures near the middle of their physiological range. For the cod this would probably lie between 5°C. and 10°C., so that it seemed unlikely that the cod in the Barents Sea were simply aggregating at a preferred temperature of 2°C., particularly as fish were frequently found in large numbers at higher temperatures but not at lower ones. It appeared that the cod had reached a limit at 2°C and it was thought that this might be the temperature limit for some physiological process.

There are few data concerning the physiological effects on marine fish of prolonged exposure to low temperature. However Doudoroff (1942, 1945) found that after a drop in temperature of 14°C., *Fundulus parvipinnis* lived for a week before finally dying, and that *Antherinops affinis* and *Girella nigricans* lived for four or five days after lesser falls in temperature. Death at low temperatures was accompanied by dehydration of the tissues in *Fundulus*, and Doudoroff (1945) showed that the time to death could be doubled by immersing *Fundulus* in a solution approximately isotonic with the blood. Very similar results were achieved with young freshwater salmon *Oncorhynchus nerka*, by Brett (1952). Both authors concluded that death after prolonged exposure to low temperatures was related to osmotic imbalance and Doudoroff suggested that failure of the osmoregulatory mechanism was the cause of death. This agrees well with the observations of Wikgren (1953) that subjection of freshwater carp, *Cyprinus carassius*, and lampreys, *Petromyzon fluviatilis*, to low temperatures resulted in excessive loss of ions by the fish.

Marine teleosts maintain their osmotic balance by absorbing sea-water from the gut and selectively excreting salts from the gills, they also gain water from their food (Krogh 1939). The hypothesis was advanced that the 2°C limit for the cod in the Barents Sea might well be acting as an incipient lethal temperature, possibly operating through the breakdown of this mechanism of osmotic regulation, and that the few cod caught in water below 2°C would be in a state of osmotic imbalance. If fish remained at these lower temperatures it was assumed that they would finally die, although the results of Doudoroff and Brett showed that death might take several days to occur.

During the summer months of July to September cod are sometimes caught in large numbers at temperatures down to between 0°C. and –0·5°C., a considerable increase in range for this fish, which normally encounters a complete temperature range of only 7C°. in the Barents Sea. Any hypothesis that temperature acted upon the fish as a limiting factor had to account for the summer change in temperature range.

In fish, as in the higher vertebrates, the activities of the system of endocrine glands have been shown to modify physiological processes, and, in particular, to produce variations in the osmoregulatory ability of both marine and freshwater fish (Pickford & Atz, 1957). If low temperatures operated through breakdown of the mechanism of osmoregulation, it might be expected that the incipient lethal temperature would also tend to vary with changes in the activity of the endocrine glands and it was thought that this might explain the seasonal variations in the apparent limiting effect of temperature on the observed distribution of the cod on the Svalbard shelf.

The final hypothesis was therefore that temperature had a real effect upon the cod, acting as a physiological limit, possibly operating through the breakdown of the mechanism of osmotic regulation ; and that the temperature limit of the cod might vary during the course of the year in accordance with changes in the physiology of the fish, which were probably controlled by the activity of glands of the endocrine system.

MATERIAL AND METHODS

The cod (*Gadus morhua* L.) were caught throughout the year in the Svalbard shelf region of the Barents Sea, between latitude 73°N. and 79°N. Adult and immature fish of both sexes, varying in length from 50 to 120 cm., were used. They were taken with a standard otter trawl towed across the sea-bed for periods of up to two hours. The temperature of the water 5 m. above the bottom was measured both at the beginning and end of each trawl haul. The work was carried out aboard the Ministry of Agriculture, Fisheries and Food Research vessel *Ernest Holt* ; the ship and details of the methods routinely employed in fishing and hydrographic operations have been described by Graham *et al.* (1954).

Blood analyses

The cod from which samples of blood were taken for analysis were lively undamaged fish selected from the deck-pounds as soon as they were released from the trawl. They were killed by a blow on the side of the head and blood

was withdrawn from the heart into a heparinised all-glass syringe. Samples were then centrifuged for about twenty minutes at 2,000 r.p.m. All determinations were carried out on blood plasma, which was analysed immediately whenever possible, or stored in plastic tubes at 1–2°C.

The accuracy of the analyses was subject to a number of serious limitations. The fish may have been bruised or damaged by the trawl, although rejection of fish in poor condition would reduce this effect, furthermore it was not known how active fish were during the period of confinement in the trawl net on the sea-bed, nor the precise time for which they had been in the net. The feeding of the fish was very variable ; generally their stomachs were empty during the winter and spring, but during the summer months most fish were feeding very heavily. Some haemolysis occurred quite commonly and occasionally there was a small amount of clotting in blood samples, despite heparinization. Working conditions when collecting blood samples were not the controlled conditions of a physiological laboratory ashore, and there can be no doubt that all of these factors, together with the differences in sexes and maturities of the fish, must have increased the variation between the results of analyses from individuals, but as the fish were considered in groups the variation within each group would tend to be the same at any one time of the year.

Chloride and freezing-point determinations were carried out in the ship's laboratory. Chlorides were estimated by mercuric nitrate titration (Schales & Schales 1941 ; Asper, Schales & Schales, 1947) using special lighting conditions to determine the end-point precisely. Two, or three titrations were performed on each sample. Freezing point determinations were made with a Beckmann apparatus for small quantities of liquid, the thermometer being read to 0·001°C., using a thermometer magnifier. The results were corrected for undercooling.

For sodium and potassium determinations the plasma proteins were centrifuged out after precipitation with 10 per cent trichloroacetic acid, a volume of the centrifugate was then stored in a plastic tube sealed with polythene tape, and deep frozen at –23°C. to await analysis with an E.E.L. flame-photometer in the shore laboratory.

Histological techniques

Specimens for histological examination were dissected from the cod as quickly as possible after the blood sample had been taken and were fixed in Bouin's fluid, unless otherwise stated. The fixed tissues were dehydrated and embedded in paraffin-wax aboard the ship.

Sections of the thyroid gland were stained with haematoxylin and eosin, and with Heidenhain's Azan. An estimate of the activity of the gland was obtained by measuring the height of the follicular epithelial cells ; the reliability of this method for fish has recently been reaffirmed (Pickford, 1953). Since individual follicles of the gland showed different degrees of activity, 200 cells were measured in each fish to overcome this variability and the result was expressed as the mean follicular cell height. In estimating the activity of the thyroid gland at any time of the year a sample of twelve fish was measured to achieve an average figure. The staining reaction of the colloid

in Azan sections gave additional evidence of the activity of the thyroid ; resting colloid appeared hyaline and stained deep red, whilst in active glands the colloid was granular and stained blue.

Sections of gill tissue were stained for mucous by the Periodic acid-Schiff reaction (P.A.S.). Adjacent sections were stained by a number of techniques to distinguish the " chloride secretory cells " (Keys & Willmer, 1932), the most satisfactory of these was found to be the Mallory trichrome method, by which these cells were stained strongly with acid fuchsin. Copeland's modification (1948) of the Leschke chloride test, using silver deposition, was used to identify the chloride cells, but could not be used routinely due to the variable success experienced with the method.

RESULTS OF PLASMA ANALYSES

The results of plasma analyses for chloride, freezing point depression, sodium and potassium for three typical cruises are presented as scatter diagrams, Figs. 1 and 2, in which the results for fish caught at temperatures above and below 2°C. are shown by separate symbols.

Fig. 1 gives the results obtained from two cruises when the fish were limited in their distribution by the 2°C. isotherm.

It is apparent that the values for fish caught below 2°C. are higher on the average than those caught above 2°C., the low temperature values extending from within the range for warm water fish to values, up to 25 per cent higher. It is considered that the values for fish caught above 2°C. represent the range for fish in a normal condition of salt balance (under the conditions of collection described), since no differences were found between the salt content of the blood of fish caught at 2°C. and at 4°C. or 5°C. But from the marked increase in the values for the blood from cod caught at temperatures below 2°C. it is apparent that these fish were in a state of osmoregulatory imbalance. This is emphasised by the fact that many of the cold water fish were caught at temperatures only half a degree below 2°C.; only 10 per cent of the cold water fish in the figure were caught at temperatures below 1°C and none below 0°C.

The results from a cruise made during the summer feeding season, when cod may be found in large numbers at temperatures down to just below 0°C., are given in Fig. 2 ; it can be seen that the range of the results for fish caught both above and below 2°C. is the same.

Not all the fish caught below 2°C. during the winter and spring showed abnormally high blood salt values. Apart from individual physiological variations, a number of factors can account for this. Some fish were caught at temperatures only a fraction below 2°C., and any fish caught on trawl hauls beginning in cold water but ending in warmer water, possibly just above 2°C., were classified as ' caught below 2°C.' Again, a time factor was involved and if fish had been in cold water for only a short period, little change in the blood salts might be detectable. This was shown by the fact that the fish with the highest blood salt contents were not necessarily those caught at the lowest temperatures but were those which were caught at a considerable distance inside the cold water, and which had therefore probably been at temperatures below 2°C. for a long time.

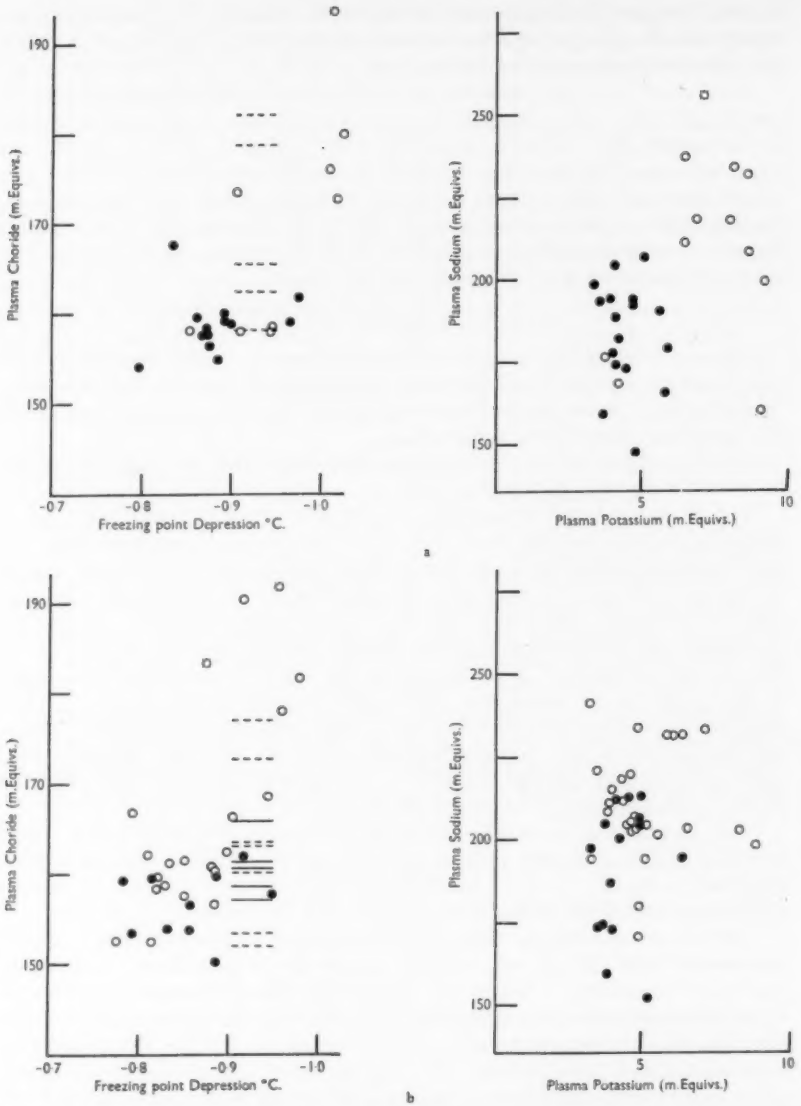

Fig. 1.—Scatter diagrams showing the results of plasma analyses for cod caught on two cruises in winter and spring. (a) November–December, 1954 ; (b) early June, 1955. ● = Cod caught above 2°C.; o = Cod caught below 2°C. In some cases there was insufficient plasma for a freezing point determination and only the chloride analysis was carried out ; these are indicated as horizontal lines on the figure.

——— = Cod caught above 2°C.; – – – = Cod caught below 2°C.

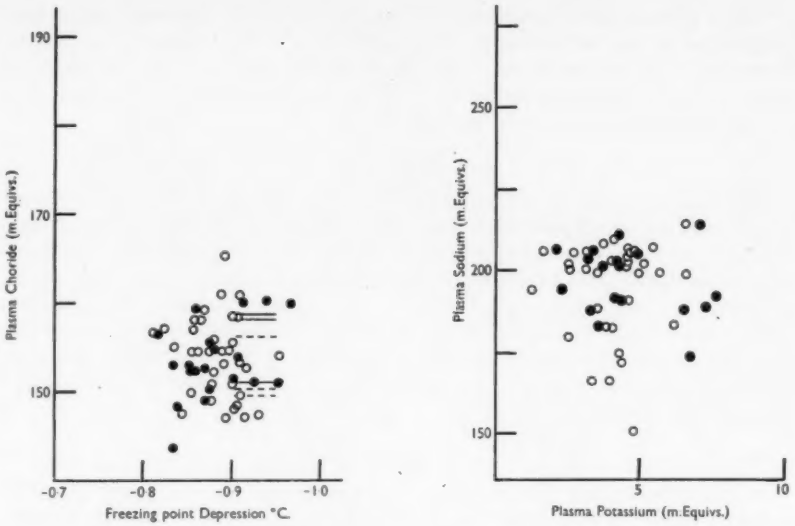

Fig. 2.—Scatter diagrams showing the results of plasma analyses for cod caught on a summer cruise, late July, 1955. ● = Cod caught above 2°C.; o = Cod caught below 2°C. In some cases there was insufficient plasma for a freezing point determination and only the chloride analysis was carried out ; these are indicated as horizontal lines on the figure.
——— = Cod caught above 2°C.; – – – = Cod caught below 2°C.

Altogether analyses have been made on eight cruises at different times of the year, involving 160 cod caught below 2°C. and 130 cod caught above 2°C. The results (summarised in the Table) show that there is no difference in the salt content of blood taken from cod above and below 2°C. in July and September, whereas markedly higher values were obtained from fish caught below 2°C. between October and June. It is apparent that during the latter period

Time of year	State of salt regulation in cod caught below 2°C.
October, 1954	Imbalance
October, 1957	Imbalance
Nov.–Dec., 1954	Imbalance
December, 1957	Imbalance
April, 1954	Imbalance
June, 1954	Imbalance
July, 1954	Normal
September, 1957	Normal

temperatures below 2°C. exert a real effect upon the physiology of the cod, disturbing the normal balance of salts in the blood, indicating the lack of adequate powers of osmotic regulation. During the summer months the cod are able to retain their normal osmotic control at these low temperatures.

These physiological results closely parallel the earlier observations on the distribution of the cod on the Svalbard shelf (Lee, 1952, Graham *et al.*, 1954) summarised by Lee (1956) as.. " paying catches of cod . . . are not taken in water with a temperature below 2°C. except in July to September when the cod sometimes enter very cold water to feed ".

GILL CYTOLOGY

The high salt content found in the blood of cod caught below 2°C. from October to July, could have been due to the primary effect of low temperatures on the mechanism of osmotic regulation, or the secondary effect of a primary failure elsewhere. It is generally accepted that the main site of salt regulation in teleosts is in specialised cells of the gills—the chloride secretory cells (Krogh, 1939). A histological examination of the gills was therefore made to ascertain whether differences could be observed in the gills of fish from warm and cold waters, which might indicate that the mechanism of salt balance was directly effected by low temperatures.

Chloride secretory cells

A number of staining techniques were employed to study the chloride secretory cells. The silver nitrate test modified by Copeland identified these cells, but in our hands the results have so far been too capricious for the method to be used to study changes in secretory activity. With Mallory's trichrome method the chloride cells were found to be strongly fuchsinophilic, staining a deep, dull pink.

Apart from their staining properties the chloride secretory cells could be identified by their size and position on the gill leaflets. In many respects they resembled the cells described in the gills of the eel (*Anguilla vulgaris*) by Keys & Willmer (1932). The chloride cells were much larger than the respiratory epithelial cells, their shape was rather irregular and more variable than in the eel, some being ovoid, others columnar. The cytoplasm had a fine granular appearance the granules staining intensely with acid fuchsin ; the nucleus was large and spherical and was usually placed centrally, or more basally in the columnar cells. The nucleus had a fine network of chromatin with occasional thickenings, and stained only faintly with Mallory ; a single nucleolus was sometimes apparent. These cells occurred mainly at the base of the gill leaflets, although isolated cells occurred on the leaflets themselves. Characteristically the bases of the chloride cells were applied to the membrane of the blood vessel in the basal region of each leaflet, although some cells also occurred separately at a small distance from the blood vessel. From four to nine chloride cells generally occurred between each pair of leaflets. The diameter of the chloride cells was about 8 μ and their nuclei 4 μ (Fig. 3 and Pl. I).

Examination of the gills of cod caught in water below 2°C., which were known to have abnormally high quantities of salt in their blood, revealed a number of differences in the condition of the chloride secretory cells. Perhaps the most striking change was in the numbers of these cells ; in the gills of the cod from cold water there were two to three times as many chloride cells between each pair of leaflets as in fish caught above 2°C. The chloride cells

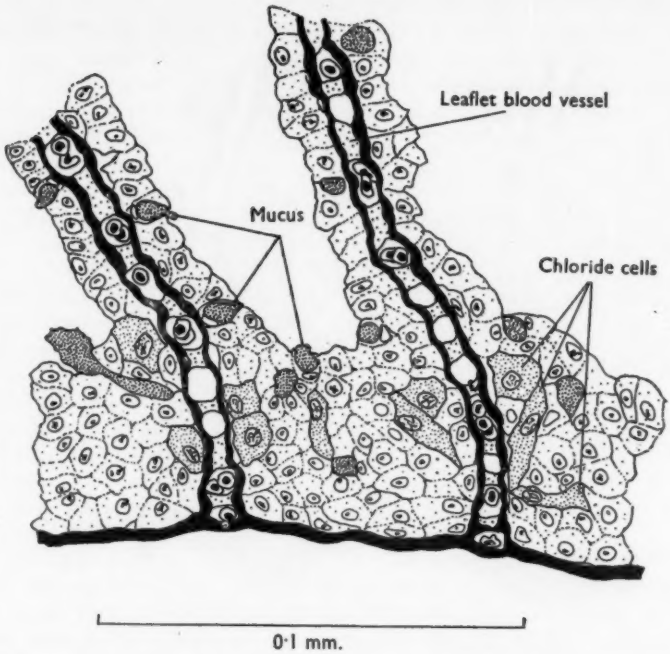

Fig. 3.—Diagram of a section through the gill leaflets of a cod from water above 2°C. The chloride secretory cells are large fuschsinophilic cells, with large nuclei, found in the basal region of the gill leaflets, generally lying close to the leaflet blood vessel. Mucous cells are also shown.

extended from the basal region of the leaflet up into the leaflet itself in large numbers ; usually they were packed side by side along the leaflet blood vessel. These cells in cold water fish generally increased in size to an average diameter of 12–16 μ. No changes were seen in the nucleus of the chloride cells, but the cytoplasm stained more deeply with acid fuchsin and the granulation was coarse and conspicuous. Copeland (1948) and Getman (1950) described the development of small pits " excretory vesicles " at the free end of the chloride secretory cells in sea water adapted *Fundulus heteroclitus* and *Anguilla rostrata*. Vesicles were not observed in individual cells of the cod, but in the gills of fish caught in cold water two or three chloride cells were frequently found grouped around a common " vacuole " (Fig. 4 and Pl. 1). These " vacuoles " may be analagous to the "excretory vesicles" of *Fundulus* and *Anguilla* ; concentrations of halides were demonstrated in some of them by the Leschke chloride test, although this was not always the case.

The greater numbers of chloride secretory cells, their increased size, and the marked granulation of their cytoplasm, together with the occurrence of " excretory vacuoles ", which were observed in the gills of cod from cold water,

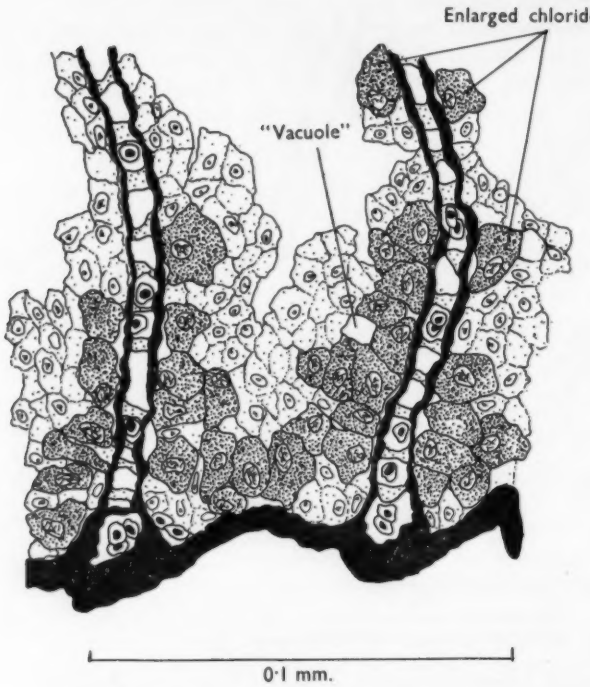

Fig. 4.—Diagram of a section through the gill leaflets of a cod from water below 2°C. The chloride secretory cells are larger and more numerous than in fish from above 2°C. Two "excretory vacuoles" are shown.

all suggest a state of increased secretory activity. If this interpretation is correct, the hyperactivity of the chloride cells may be a compensatory response of the mechanism of salt balance to reduce the abnormally high salt content of the blood. In view of the absence of signs of pycnosis, breakdown, or exhaustion of the chloride cells it seems that the primary site of the action of low temperatures must be elsewhere.

Examination of the gills of cod caught in waters above and below 2°C. in the summer months of July to September did not show any increased activity of the chloride secretory cells in cold water fish, except for some fish caught between +0·26°C. and −0·14°C. In this latter sample the chloride cells of a few fish showed some evidence of an increase in secretion although this was not marked, nor found in all fish in the sample ; it therefore seems likely that these cod were beginning to approach their summer incipient lower lethal temperature just below 0°C., although plasma analyses showed that they were not yet in a state of salt imbalance.

Mucous cells

Mucous stained bright purple-pink with the P.A.S. technique. In cod from warm water mucous filled goblet cells were spaced fairly regularly along each gill leaflet, and four to six mucous cells occured in the basal region between each pair of leaflets. Often the space between the leaflets also contained free mucous. The gills of cod caught below 2°C., in a state of salt imbalance, exhibited a marked depletion of mucous which was often absent from the leaflets, or reduced to one or two cells, depletion also occurred in the basal region between leaflets. The few goblet cells which were seen on the leaflets of cold water fish were usually atrophied and discharging their contents (Pl. 1). The depletion seen on the leaflets also extended to the region of the gill-rays, although to a lesser extent, but even in this mucous rich area the number of mucous-filled goblet-cells was reduced to about one-third of their normal level.

Mucous performs an osmotic function in fish decreasing the permeability of the external coverings to ionic exchange (Pickford & Atz, 1957). When the gills are depleted of mucous, there is only a single layer of respiratory epithelium between the seawater and the blood, and it seems probable that salt may enter the circulation more rapidly in this region*. The gills offer the greatest area of the body surface of the fish to the external environment and this increase in the osmotic load on the fish could therefore be very considerable. In this way, the dislocation of mucous production, either as a primary or a secondary effect of low temperature might well be the cause of the increased salt content found in the blood of the fish caught in cold water between October and June. Marked mucous depletion was not observed in the gills of fish caught in cold water during the summer months, and analyses of the blood of these fish showed no evidence of osmotic imbalance.

Little is known of the factors controlling mucous production in teleosts, but Burden (1956) reported that when hypophysectomised *Fundulus heteroclitus* were kept in fresh water, the mucous cells of the gills atrophied and decreased in number this was accompanied by a fall in blood chlorides, and finally followed by death of the fish. The mucous cells could be restored to their normal condition and number by injecting the hypophysectomised fish with *Fundulus* pituitary brei. Although Burden observed atrophy of the mucous cells of hypophysectomised *Fundulus* kept in sea-water, no decrease in their numbers occurred, and, perhaps surprisingly, the blood chloride did not rise.

SEASONAL CHANGES IN THE EFFECTS OF LOW TEMPERATURES

The results of plasma analyses showed that the effects of low temperatures varied during the course of the year. As part of the original hypothesis it was suggested that such variations might be linked with changes in the activity of glands of the endocrine system. This suggestion has recently received

* A high salt content in the blood has been taken throughout the paper as indicating a breakdown of the mechanism of osmoregulation This may have been due to entry of salt into the circulation more rapidly than it could be excreted but dehydration may have also played a part; to determine this attempts were made to analyse the water content of the muscles but facilities on board the vessel proved inadequate.

considerable support from the report of Hoar (1955) that marked seasonal variations occur in the ability of goldfish to withstand low temperatures, although kept under constant nutritional and thermal conditions. Hoar (1956) further showed differences in the low temperature resistance of two groups of goldfish maintained under the same conditions but with one group receiving twice the daily period of illumination of the other group, and he concluded that the differences were due to photoperiodically controlled changes in endocrine physiology. Hoar (1955) obtained some support for this suggestion by demonstrating alterations in the lower lethal temperatures of goldfish kept in solutions of thyroid drugs.

It seemed unlikely that gonadal secretions, or the onset of maturation, were responsible for the observed variations in the effects of low temperatures, since the effects appeared to be the same in both immature and maturing cod. Of the other endocrine glands the thyroid seemed most likely to be linked with these changes as it had been associated with teleostean osmotic regulation in a number of earlier studies (reviewed by Hoar (1957), and Pickford & Atz (1957)).

Azan staining clearly distinguished " resting " and " active " colloid in sections of the thyroid gland. As in other teleosts, active secretion was accompanied by granulation and vacuolation of the colloid, and occasionally the follicular walls broke down and the colloid was invaded by leucocytes. A quantitative estimate of activity was achieved by measuring the height of the follicular epithelial cells. The thyroid became active in both adult and immature cod at the end of September when the mean follicular cell height increased from a resting level of 6·8 μ and 6·4 μ to 8·4 μ and 8·1 μ respectively. Thereafter the cell height increased slightly during the winter in the immatures and considerably in the adult cod, and activity continued until March–April, when the gland entered a resting condition until the following autumn.

The cod lose their ability to survive in water below 2°C. at the beginning of October, when the thyroid gland has become active in both immature and adult fish, but they do not regain the ability to enter the colder waters until some two months or more after thyroid activity has ceased. It is possible that the thyroid hormone is lost only slowly from the blood and continues to circulate for some time after the gland has entered the resting condition. Thus it may take three weeks for all thyroxine to disappear from the tissue of thyroidectomized rats, and Matty (1954) has shown the presence of protein bound iodine in the blood of dogfish *Scyllium canicula* L. some six weeks after surgical thyroidectomy.

The continued presence of thyroid hormone in the blood for some weeks may be involved in the inability of the cod to survive in cold water after the thyroid becomes inactive, but the very poor nutritional state of the fish at this time of year is probably a complicating factor. In the Svalbard region the cod feed very little, if at all, during the winter and spring, and by the end of May they are thin, weak, and very dull in colour, the skin is flaccid and often feels slightly sandy or roughened, the liver is very small and highly discoloured with bile pigments, and the fish appear to be in a state of starvation. The majority of these fish die very rapidly on deck and even when placed immediately in tanks of sea-water. The fish caught five weeks later, in July, when

they are feeding heavily, provide a marked contrast, being firm and smooth to the touch, glistening and brightly coloured, and very lively indeed on deck. The cod are not found in numbers in water below 2°C. until the summer feeding has begun, fish on the east side of the Svalbard shelf, where plankton production begins earlier (Marshall, 1955), start to feed before the cod on the western side of the shelf and have been found to enter the cold water mass earlier in the summer. So it seems that the cod must begin to feed heavily before they regain the ability to withstand temperatures below 2°C., and that prior to this the starving cod are probably in too poor condition. Results of experimental work which have shown that differential feeding, and starvation can decrease the resistance of fish to low temperatures (Ellis, 1947, Hoar & Cottle, 1952, and Irvine, Newman & Hoar, 1957) support this conclusion. Feeding in itself would tend to reduce the osmotic load on marine fish since metabolic water would be produced by the combustion of foodstuffs.

In the autumn the cod lose the ability to survive in water below 2°C., although they are in good condition after the heavy summer feeding, and are still feeding well; this cannot therefore be linked with starvation. It seems that changes in endocrine physiology are most likely to be involved. That endocrine changes occur at this time has already been demonstrated for the thyroid gland. However, comparisons of the thyroids of cod caught above and below 2°C., both in seasons when the gland was active, and when resting, have not shown significant differences. These results neither prove, nor disprove, that the thyroid gland itself is directly involved in the change in the resistance of the cod to low temperatures. Other endocrine glands may be responsible—the pituitary has already been mentioned in connection with mucous secretion, and further histological studies are being carried out, but the final understanding of the mechanism of the seasonal changes in temperature limit can only result from experimental studies with hormones on cod at low temperatures.

GENERAL DISCUSSION AND CONCLUSIONS

A number of investigators have reported apparent relationships between the distribution of cod and water temperatures. But in referring to the lower temperature limits to the distribution of the fish, reports are confused by the occasional occurrence of a few fish at very low temperatures, and seasonal variations in limits are seldom taken into account. In the Barents Sea the results of work carried out on the *Ernest Holt* to study the distribution of the cod and temperature on the Svalbard Shelf suggested that 2°C. might act as a temperature limit to the fish during winter and spring, but not during the period July to September. Saetersdal (1956) has also shown that in the south eastern Barents Sea, where temperature gradients are not so steep, the cod do not penetrate far into the cold water areas, and that on occasions the distribution of the fish is described in some detail by sea-bottom isotherms (although he suggests that 1·5°C. is important, rather than 2°C.). In the central areas of the Barents Sea Maslov (1944), quoting Milinsky, also reported that in 1934 and 1935 the distribution of the catches of the Russian trawler

fleet followed the spreading of warm water north east and seemed to be related to the movement of the 2°C. isotherm.

The close fit between the observed temperature limits to the distribution of the cod in the Svalbard region, and the physiological results presented in this paper afford evidence that the relationship with temperature is not coincidental and it seems likely that the cod avoid water below 2°C. (except during the summer months), although nothing is known of the behavioural mechanism of this avoidance. The heavy concentrations of cod sometimes found in the region of the 2°C sea-bed isotherm may be caused by the interplay of a number of factors but temperature appears to play a major rôle; if the cod avoided water below 2°C but their movement was unrestricted by the higher temperatures encountered in the Barents Sea (up to 6°C), then they would tend to accumulate in the region of the cold front.

It is probable that numbers of cod wander into, or are trapped in cold Arctic water each year, and that unless they re-enter warmer waters, they die under these conditions. Catastrophic mortalities may occur periodically. Thus Thompson (1943) reported twenty miles of dead cod in the Gulf of St. Lawrence in 1934, and concluded that the fish had been killed by encountering very cold Arctic water. Dannevig (1930) and Johansen (1929) have also reported the death of cod off the Scandinavian coasts in severe winters. During the cold winters of 1929 and 1947 the occurrence of large numbers of dead fish, including cod, was reported by fishing vessels in the North Sea, and the distribution of the dead fish has been described in conjunction with available hydrographic data (Lumby & Atkinson, 1929, and Simpson, 1953). From the data for 1929 it is apparent that a majority of the dead fish were found at positions where it is unlikely that the temperatures fell below 1·5°C. Similarly Simpson reported that in 1947 dead fish were found over a wide area where temperatures could never have fallen below 1·5°C., indeed some dead cod were found where it was unlikely that temperatures were ever less than 2·5°C. Simpson's paper is of particular interest as trawlers were reported to be making large catches of cod which were concentrated along the edge of the cold water mass, reproducing in some detail the conditions frequently encounted at Svalbard.

At this point it is relevant to discuss the recent studies of Scholander *et al.* (1957) on the blood of Arctic fishes. They observed that the blood of fish caught during both summer and winter in water of −1·7°C. at the bottom of Hebron fjord, Labrador, had a freezing point of −0·9°C. and concluded that the fish could withstand the low temperatures, and were living in a permanent state of supercooling. They found that these fish did not freeze in sea-water of this temperature unless they were seeded by being brought into contact with ice, when freezing occurred rapidly. The blood of other species of fish caught in the surface waters at 4°C. during the summer months also froze at −0·7 to −0·9°C., but when the same fish were caught in the upper waters during the winter, when water temperatures had fallen to −1·5°C., it was found that the freezing point of their blood had fallen to −1·3°C., to −1·6°C. Scholander *et al.* suggested that this fall in freezing point of the blood, which was accompanied by a rise in chloride content, was an adaptation to life at these low

temperatures, in the neighbourhood of ice which might otherwise have brought about freezing of the fish. That would be surprising in view of the relative constancy of internal milieu in the vertebrates. However, the results of the analyses given above for cod caught below 2°C. during the winter and spring show similar changes, although of lesser magnitude, to those reported by Scholander, despite the fact that none of these cod were caught at temperatures below the normal blood freezing point of −0·8°C.; indeed all of the cold water cod were caught at temperatures between 2°C. and 0°C., well above this freezing point. Although these may be two different cases, it seems possible that the low freezing points observed by Scholander in the analyses of blood from fish caught in the upper waters of Hebron fjord at −1·5°C., might have been due to the fish being in a state of osmotic imbalance at this low temperature, particularly as the blood analyses for the same species during the summer months yielded values similar to those commonly reported for other marine teleosts.

The cod fishery on the Svalbard shelf has been subject to considerable periodic fluctuations, so that although there were rich catches of cod at Spitzbergen between 1873 and 1882, the fish were absent from the region in any sustained numbers for the following forty years. Since 1928, the shelf has been a common fishing ground for vessels of the British distant water trawling fleet, which at present catch between 50,000 and 100,000 tons of cod there annually. The recurrence of cod on the Svalbard shelf, like the extension of other Arctic fish stocks, has been associated by a number of authors (Berg, 1935; Jensen, 1939 ; Taning, 1953) with the pronounced climatic amelioration in the Arctic in recent decades. In the cod stock of the Barents Sea two changes have occurred ; an extension to the north and east of the geographical distribution of the fish, and an increase in size of the stock. Climatic temperature changes have been involved, and the northward extension of many warm water species of marine animals throughout the Arctic regions suggests that a corresponding rise in sea-temperature may have taken place. It is therefore pertinent to enquire whether the changes in the cod stock of the Barents Sea are in any way connected with the limiting effects of low temperature upon the fish.

It seems fairly well established that an actual extension in the distribution of the cod occurred on the Svalbard shelf. The grounds were well known to Norwegian fishermen in the nineteenth century and yet a fishing expedition in the summer of 1883 caught only three cod ; subsequent expeditions were also unsuccessful until 1923, when the cod began to reappear (Jensen, 1939), whereas cod can now be caught over almost all the shelf during the summer. In other areas of the Barents Sea Berg (1935) and Lundbeck (1954) have also shown that cod, and other warm " Atlantic water " fish, occur over large areas where they were rare or unknown for many years previously. Very similar changes have been reported for Greenland.

The cod is limited in its distribution by temperatures below 2°C. for most of the year in the Svalbard region, and therefore the geographical extension of the stock since the middle twenties might be explained if it could be shown that the position of the 2°C. bottom isotherm had receded to the north and east in the Barents Sea during the same period. Unfortunately, few useful hydrographic data are available for the area during the supposed colder period when

the cod were absent. Studying the distribution of the bottom living animals in Svalbard waters, Blacker (1957) has shown that a number of benthic species are restricted to the Atlantic or to the Arctic water masses, which he described as having temperatures above 2·5°C., and 1°C. or less respectively. Records for fifty years previous to 1931 show that the characteristic Atlantic warm water species were restricted to an area west of Bear Island, south of 75°N. Lat. Blacker has demonstrated that since 1931 there has been a marked extension of Atlantic species to the east, and to the north, along the west side of Spitzbergen, with a corresponding decline in Arctic water species over the same area, indicating a great increase in the area of the bottom over which warm Atlantic conditions predominate. The correlation established by Hill & Lee (1958) between the strength of the southerly winds and the volume transport in the West Spitzbergen current, together with the fact that the northward transport of air from the North Atlantic has increased during the present climatic fluctuation, would also suggest that there has been an increase in the Atlantic influence in the Svalbard region. It therefore seems probable that a greater area of this shelf is covered by Atlantic water and that the extension in range of the cod could be due to the displacement of the position of the limiting sea-bed isotherms.

The successful survival of a year class of the Barents Sea cod stock is determined during the drift of the pelagic stages from the spawning area to the nursery grounds, or immediately after the young fish have settled on the bottom (Wiborg, 1957). Hill & Lee (1958) have suggested that the recent increase in the size of this stock might be due to the increase in the volume transport of the West Spitzbergen current in recent years, causing the larvae to be carried to the Svalbard nursery grounds more rapidly, thereby allowing increased survival. But in addition, it seems quite probable that low temperatures may have a limiting effect upon the larvae and O-group cod, as they have on the older cod, and in such a case the increase in the cod stock might also be accounted for, at least in part, by there being a greater area of the shelf feeding grounds over which temperatures were above the lethal threshold of the young cod, allowing successful settlement on the bottom and increased survival. This is supported by the report that temperatures below 1·8°C. are lethal to cod larvae (Taning, 1953) and by the observations of Hermann at Greenland (Hachey, Hermann & Bailey, 1954) that in years when the temperature was below 1·8°C. on the Fyllas bank, where the cod larvae were found, only poor year classes survived, but when temperatures were above 1·8°C. much stronger year classes resulted. However cod larvae from the Icelandic cod spawning may be carried to Greenland by the Irminger current and it is possible that the temperature relationship described for the Fyllas bank could indicate an increased transport of cod larvae from Iceland in this warm current to augment the Greenland stocks, rather than a direct effect of low temperatures on the survival of cod larvae.

It may be concluded that, coincident with the recent climatic fluctuation in the Arctic, there is some evidence of an increased Atlantic influence on the Svalbard shelf, as suggested by the recession of the ice limit (Ahlmann, 1949), by the probable increase in the volume transport of the West Spitzbergen

current (Hill & Lee, 1958), and by the marked spread of bottom living animals characteristic of Atlantic water, accompanied by the decline of Arctic species (Blacker, 1957). Blacker has suggested that Atlantic water now covers a much greater area of the bottom, and if this is accepted, it is apparent that considerable areas of this rich feeding ground, previously covered by water which was too cold to allow survival, would be opened to all stages of the cod. This change could account for the extension in geographical distribution of the cod, and may also have played a part in the coincident increase in size of the stock.

SUMMARY

The cod (*Gadus morhua* L.) in the Barents Sea often appeared to be limited in their distribution by low temperatures. The effect of temperature seemed to be a direct one, although the limiting temperature varied during the course of the year. It was postulated that low temperature was acting as a physiological limit, probably operating through the breakdown of the mechanism of osmotic regulation, and that the temperature limit of the cod might vary during the course of the year, in accordance with endocrinally controlled changes in physiology.

Analyses of the blood of cod caught above and below the limiting temperature, confirmed the hypothesis. The fish from low temperatures were in a state of osmotic imbalance having a high blood-salt content. The physiological limiting temperature was found to be 2°C. from October to June, but fell to below 0°C. during the summer. Histological examination of the gills of cod caught below the limiting temperature showed depletion of mucous and an increase in the number and size of the chloride secretory cells. Mucous depletion would permit more rapid entry of salts from the environment into the circulation, and the changes in the chloride cells suggested an increase in secretory activity as a compensating response to the raised level of salt in the blood.

The thyroid gland became active in the autumn, coincident with the increase of the limiting temperature to 2°C., but the gland re-entered the resting condition in the spring several weeks before the limit reverted to the summer level. In the spring the cod had not fed for some months and starvation may have been the complicating factor. The cod did not regain their ability to enter water down to 0°C. until the summer feeding had begun.

The physiological results described above closely paralleled observations on the distribution of the cod with respect to temperature, and provide evidence of the direct influence of low temperatures upon the fish. The stock of cod in the Barents Sea has undergone changes in geographical distribution and in population size since the mid-nineteenth century and these have been discussed in the light of the present results. It has been suggested that the direct effects of low temperature upon the fish, in combination with the hydrographic changes which have occurred during the same period, might account for a part of both the change in the size and the distribution of this stock of cod.

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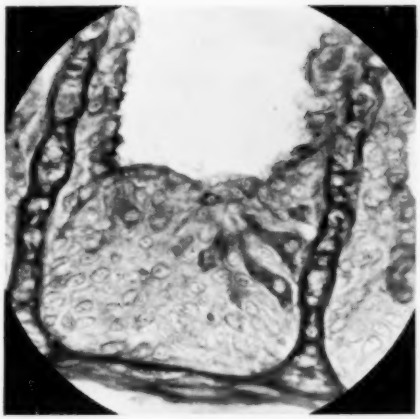
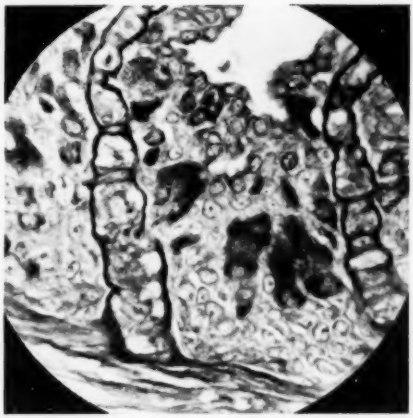
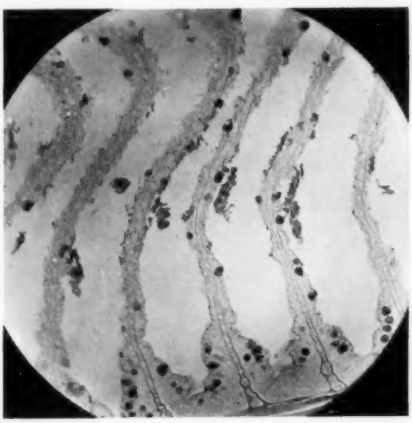


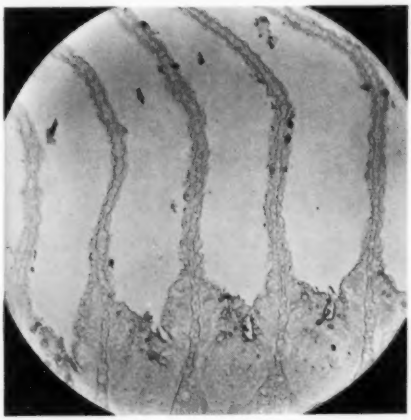
1. Chloride secretory cells in a section of the gill of a cod from water above 2 C°. Mallory modification.



2. Chloride secretory cells in a section through the gill leaflets of a cod caught below 2° C. Mallory modification.



3. Mucus in the gills of a cod from water above 2° C. P.A.S.



4. Mucus in the gills of a cod from water below 2° C. showing mucus depletion. P.A.S.

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PLATE I.

Fig. 1.—Chloride secretory cells in a section through the gill leaflets of a cod from water above 2°C. (cf. Text-fig. 3). Mallory modification.

,, 2.—Chloride secretory cells in a section through the gill leaflets of a cod from water below 2°C. (cf. Text-fig. 4). Mallory modification.

,, 3.—Mucous in the gills of cod from water above 2°C. P.A.S.

,, 4.—Section of the gills of cod caught below 2°C. showing mucous depletion. P.A.S.

A STUDY OF THE EGG SHELLS OF RATITE BIRDS

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(With 3 plates and 12 figures in the text)

CONTENTS

	Page
Introduction	202
Terminology	202
Materials	203
Methods	205
General	205
Chemical analysis	205
Membrane protein	205
True shell nitrogen : total, soluble and insoluble	205
Inorganic constitutents	205
Histochemical studies	206
Plastic embedding techniques	206
Porosity	207
Thickness	207
Results : General	207
Shell thickness	207
Porosity	207
Chemical analysis	208
Results : Individual ratites	211
Emu	211
General appearance	211
Plastic embedded sections	213
Histological sections	215
Cassowary	217
General appearance	217
Plastic embedded sections	218
Histological sections	219
Rhea	219
General appearance	219
Plastic embedded sections	220
Histological sections	222
Ostrich	223
General appearance	223
Plastic embedded sections	223
Histological sections	225
Kiwi	226
General appearance	226
Plastic embedded sections	226
Histological sections	228
Tinamou	229
General appearance	229
Plastic embedded sections	229
Histological sections	230

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CONTENTS (continued)

	Page
Aepyornis	230
General appearance	230
Plastic embedded sections	230
Histological sections	231
Moa	232
General appearance	232
Plastic embedded sections	232
Histological sections	233
Discussion	233
General shell structure	233
Detailed shell structure	235
Formation of pores	237
Classification of the 'Ratites'	239
Summary	241
Acknowledgments	242
References	242
Explanation of plates	243

INTRODUCTION

Studies of the structure of egg shells as distinct from measurements of weight and colour, have been singularly rare and particularly is this true of studies on non-domestic species. Blasius (1867) published a paper dealing with the various parts of the egg, while between 1867 and 1894, Nathusius reported at great length on various birds' eggs and he suggested that shell structure might be of use in the classification of birds, but, apart from these early papers, there is very little information until Schmidt published a series of papers which are covered by a recent review (Schmidt, 1957).

A few writers have analysed shells and shell membranes and the work is summarised by Needham (1931) and by Romanoff & Romanoff (1949). The former, however, is correct when he says that a great deal remains to be done on the shell.

In the past few years, methods for studying chemical composition, histological and mechanical structure, as well as porosity, have been developed at Reading. It is the purpose of this paper to report the results of the application of these methods to a collection of egg shells belonging to families at one time grouped in the division Ratitae of the sub-class Neornithes.

TERMINOLOGY

A radial section of an idealised egg shell, less membranes and cuticle, is shown in Fig. 1a. The chief components are the spongy and the mammillary layers. On the outside of the spongy layer is the cuticle, if any, and on the inside of the mammillary layer would be the two membranes. The mammillary layer was so named by Nathusius (1868) and derives its name from the mammillae which form its inner surface. The knob of each mammilla is quite separate where it is attached to the membrane but, at the end away from the membrane, the knobs are fused together and cannot be separately distinguished. The mammillary layer is not, therefore, confined to the visibly separate knobs.

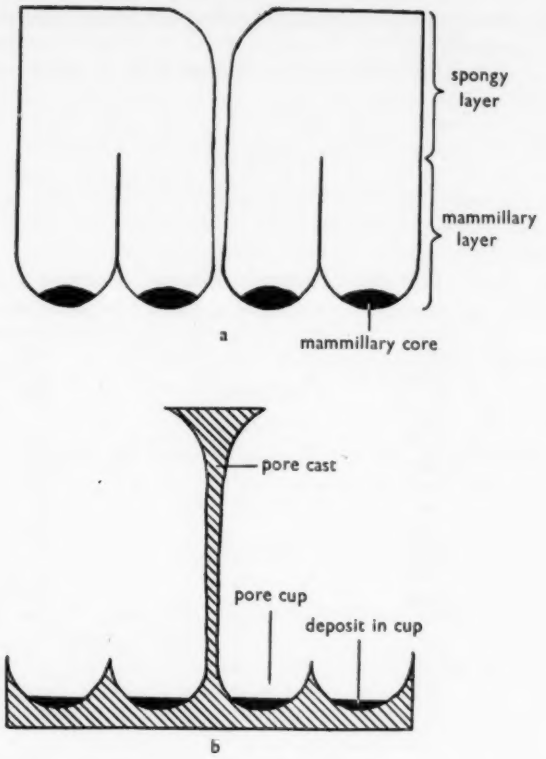

Fig. 1.—Conventional diagram showing (a) general shell structure, and (b) plastic cast derived from it.

The spongy layer was given this name by Landois (1865) because of its appearance after acid attack, but it is, normally, very compact. Organic material is spread throughout both spongy and mammillary layers, but there is an especially strong concentration of such material as a core in the inner end of each mammillary knob. The above refers to the simplest of structures; additional structural layers may be present in some shells and will be mentioned as required.

MATERIALS

At least three eggs of each of the following birds were used: ostrich (Struthionidae), rhea (Rheidae), emu (Dromiceidae), cassowary (Casuariidae), kiwi (Apterygidae) and tinamou (Tinamidae). There is considerable disagreement about including the tinamous with the ratites, but it was decided to do so here. A small piece of each of two *Aepyornis* shells was also available for some of the tests. Tyler (1957) has recently published an account of samples

from six moa (Dinornithidae) shells and, where relevant, these data will also be given for comparison.

Table 1 gives the source of supply of the eggs and the serial numbers which have been allocated to them.

TABLE 1

Source of shells and serial numbers allocated to them.

Family	Serial No.	Source	Family	Serial No.	Source
Emu	882	R	Kiwi	1272	B
	884	R		1329	B
	1208	O		1330*	B
	1333	B			
	1334	B	Tinamou	1336	B
	1335*	B		1337*	B
				1338	B
Cassowary	1273	B			
	1331*	B	*Aepyornis*	1148	?
	1332	B		1359	B
Rhea	881	R	Moa	1201	O
	883*	R		1202	O
	1326†	B		1203	O
	1327	B		1204	O
	1328†	B		1205	O
				1206	O
Ostrich	873	R			
	874	R			
	875	R			
	876	R			
	877	S			
	878	S			
	879	S			
	880	S			
	1207	O			
	1298	I			
	1300	I			

* Samples probably from incubated egg
† Green

B = British Museum
O = Otago Museum
R = Reading Museum
S = South African ostrich farm
I = Ilfracombe Zoo

All were museum samples of blown eggs, except those from the South African ostrich farm which were fresh egg shells broken and washed, and those from Ilfracombe Zoo which were received fresh and intact.

It is important to mention here that supplies of these egg shells are not easy to obtain and particularly is this so in relation to fresh samples. Furthermore, the sub-division of this material, most of which is museum material, into

species, is very difficult or impossible. However, a wide experience of many egg shells confirms that there are few, if any, species differences in relation to the characteristics to be discussed in this paper, and this is supported by the results with the tinamou shells. The species available were *Tinamus salvini* (1336), *Nothocercus bonapartei* (1337) and *Eudiania elegans* (1338), but no major differences were noted. The discussion is therefore, based on the families concerned.

Mention should be made of some samples of shell which showed a hole in the end of each mammilla. These are marked in Table 1. With most eggs this probably indicates that they have been incubated (Tyler, 1958), but with shell samples, such as moa and *Aepyornis*, recovered after many years, it is probable that true erosion has also taken place. This can often be verified because erosion may attack the outer surface as well.

METHODS

General

Pieces of untreated shell were examined under the stereoscopic microscope and particular attention was paid to the appearance of radial sections of freshly broken pieces.

Chemical analysis

Membrane protein

Boiling in 2·5 per cent (w/v) sodium hydroxide solution as recommended by Tyler & Geake (1953a) was used.

Samples which are not fresh have often lost part of their membranes, therefore this value was only determined in cases where there was absolute certainty that the membrane was fully present on the pieces analysed.

True shell nitrogen : total, soluble and insoluble

The method was that of Tyler & Geake (1953a) using 10 per cent (w/v) sodium hydroxide solution, but with the newly recommended terminology of Tyler & Simkiss (1958).

Inorganic constituents

Pieces of membrane-free shell (true shell) were dissolved in 1·0 N hydrochloric acid at room temperature and, after filtering off any organic matter, the solution was made up to volume. Sodium and potassium were determined in this solution, using an Eel flame photometer. The disodium salt of ethylenediamine tetra-acetic acid with murexide as indicator was used for the volumetric determination of calcium (Schwarzenbach, 1955). Magnesium was determined by the method of Hunter (1950) except that, after the excess of dye had been extracted with the organic solvent, a known volume was poured off into a tube containing 5 ml. of absolute ethyl alcohol.

Another piece of membrane-free shell was dissolved in 1·0 N hydrochloric acid and carbonate was then determined indirectly by estimating the residual acid. A third piece of shell was digested with a mixture of nitric and perchloric acids and phosphorus determined by the method of Hanson (1950).

All values for inorganic constituents were expressed on the basis of nitrogen-free shell.

One sample of *Aepyornis* shell was so small that none could be spared for chemical analysis.

Histochemical studies

The methods were those used by Simkiss & Tyler (1957) on the decalcified hen egg shell, except that, for disulphide groups, reducing substances were blocked with acetic anhydride before reducing the disulphide groups with potassium cyanide. The area of the section occupied by the original disulphide groups was then detected with the ferri-ferricyanide reagent. In addition, acetylated and purified Sudan black B in propylene glycol was used for fat staining on frozen gelatine sections.

Pieces of shell which had been treated with 2·5 per cent and with 10 per cent sodium hydroxide solution as mentioned under chemical analysis, were also examined histologically after decalcification.

In a further paper, Simkiss & Tyler (1958) have suggested that the organic matter of the hen egg shell contains material which can chelate with cations. The chelating power may be roughly measured using the competition between toluidine blue and cupric ions and the " elimination concentration " is the term used for this value. This measurement has been used on the shells discussed in this paper.

Plastic embedding techniques

Tyler (1956) has described two main methods for use on shells embedded in plastic. First, a radial section of the shell is exposed by filing away the plastic and this section is then etched with concentrated hydrochloric acid for a few seconds. This etching very often reveals distinct layers which are etched to differing degrees. Second, the outer surface of the shell is exposed by filing away the plastic and then the plastic block is placed in concentrated hydrochloric acid until no more carbon dioxide is evolved. This treatment produces a cavity in the plastic where the shell had previously been. The floor of this cavity gives a cast of the mammillary knobs which are on the inside of the shell and, projecting from this floor, like stalagmites, are plastic casts of the original pore channels. These structures are shown diagrammatically in Fig. 1b, which also shows their relationship to the original shell of Fig. 1a. The pore casts may be dissected off and mounted in cavity slides, but it must be borne in mind that such pore casts will only represent pores from a small area of a shell and will not necessarily be representative of the whole shell.

Plastic embedded material has also been used to give both radial and tangential sections of shell cut sufficiently thin as to be fairly transparent. These methods give essentially the same kind of section as those produced by Nathusius (1868), except that he used Canada balsam to hold the shell together when grinding.

To produce a tangential section, the method is that described by Tyler (1954), except that, when the section is finally prepared, it is fastened on to a microscope slide, ground face downwards, with DPX and examined through

the slide using transmitted light. A drop of xylene is put on to the section and on to the DPX before pressing the section firmly on to the DPX. A thin radial section is rather more difficult to produce because the shell so easily crumbles. The plastic block is first ground until a good radial section of the shell is obtained, then this block is re-embedded in more plastic. When set, grinding is commenced from the other side of the section and continued until it is transparent. The other face, being backed with plastic, helps to prevent the sampling from crumbling. Once again the sample is fastened to a slide with the ground face to the slide.

Porosity

Tyler (1945) used a method for measuring porosity based on the rate of loss of water from the fresh egg under standard conditions. All except one or two of the eggs available for this present work were blown and only a few of these were still whole ; therefore, apart from two ostrich eggs, the small number of porosity measurements available refer to blown eggs filled with water and stoppered with a small rubber stopper. The determinations were made using large desiccators instead of 1 lb. squat jars.

Thickness

Five measurements were made with a micrometer screw gauge on each of ten pieces of shell (without membrane) taken at random, and the mean of these fifty readings calculated. There was insufficient *Aepyornis* shell to provide ten pieces taken at random so a total of ten readings was taken.

RESULTS : GENERAL

Shell thickness (Table 2)

It will be seen that the samples of *Aepyornis* shell are about 3·5 mm. thick ; this is much greater than the value of approximately 2 mm. given for ostrich shells. The ostrich shells are on the average much thicker than the moa shells, whilst the rhea shells are considerably thinner, being usually less than 1 mm. Despite the corrugations on the emu and cassowary shells, it is possible to obtain consistent readings for these shells and it will be seen that the emu gives values similar but perhaps slightly thicker than the cassowary, whilst they are both slightly thicker than the rhea. Kiwi shells are, considering the size of the egg, very thin, while tinamou shells are also thin.

Porosity (Table 2)

Very few results are available but the values for the ostrich and cassowary are of the same magnitude as those for the domestic hen, namely, about 1·7–2·3 mg loss/day/sq. cm. (Tyler & Geake, 1953b), while those for the rhea are considerably higher. The emu also gives high values except for 1335 which had suffered some breakdown of the mammillary layer, but this is not necessarily the reason for a low value here. The single tinamou value is around the lower values for the domestic hen. It is important to remember that, of all these determinations, only the two values for the ostrich were determined on the fresh unopened egg.

TABLE 2

Shell thickness (μ) and porosity coefficients (mg loss/day/sq. cm.).

Family	Serial No.	Thickness	Porosity	Family	Serial No.	Thickness	Porosity
Emu	882	1141	—	Kiwi	1272	498	—
	884	1150	—		1329	343	—
	1208	1116	—		1330*	441	—
	1333	983	3·35				
	1334	1057	2·93	Tinamou	1336	250	—
	1335*	1023	0·83		1337*	198	—
					1338	216	1·72
Cassowary	1273	1087	2·35				
	1331*	903	—	*Aepyornis*	1148	3469	—
	1332	1064	2·16		1359	3508	—
Rhea	881	883	—	Moa†	1201	1420	—
	883*	1021	—		1202	1120	—
	1326	895	3·51		1203	1400	—
	1327	925	—		1204	1440	—
	1328	864	3·49		1205	1460	—
					1206	1340	—
Ostrich	873	1811	—				
	874	1881	—				
	875	1970	—				
	876	1868	—				
	877	1737	—				
	878	2038	—				
	879	2137	—				
	880	1914	—				
	1207	1922	—				
	1298	1850	2·06				
	1300	1887	2·10				

* Samples probably from incubated egg.

† Some pieces were eroded and the values given represent the thickest piece available for each bird. The value for 1202 is not reliable because all pieces were very badly eroded.

Chemical analysis (Tables 3 and 4)

It is quite clear that, on the basis of percentage constituents in the nitrogen free shell (Table 3), there are no real differences in the contents of calcium, carbonate, potassium, or sodium. However, on the basis of mean values, there are considerable variations in the magnesium content; the single sample of *Aepyornis* shell analysed gave 0·25 per cent, whilst, at the other end of the scale, the emu and cassowary gave 0·07 per cent and 0·08 per cent respectively. Again, there were considerable variations for the percentage of phosphorus, the tinamou gave a mean value of 0·28 per cent, whilst, at the other extreme, the ostrich gave 0·06 per cent and the *Aepyornis* 0·03 per cent. It should also be noted that the individual kiwi shells gave widely differing values. Thus only magnesium and phosphorus appear to vary.

TABLE 3

Chemical analysis. Inorganic constituents expressed as percentage of nitrogen-free shell.

Family	Serial No.	Ca	Mg	K	Na	CO_2	P
Emu	882	39·2	0·05	0·03	—	43·8	0·10
	884	39·3	0·11	0·02	—	43·7	0·10
	1208	39·4	0·06	0·03	—	43·6	0·10
	1333	39·5	0·08	0·02	0·04	43·4	0·11
	1334	39·4	0·08	0·02	0·03	43·4	0·13
	1335*	39·5	0·06	0·02	0·04	43·5	0·14
Mean		39·4	0·07	0·02	0·04	43·6	0·11
Cassowary	1273	39·1	0·09	0·03	0·04	43·6	0·10
	1331*	39·2	0·08	0·02	0·04	43·7	0·20
	1332	39·1	0·08	0·02	0·04	43·7	0·12
Mean		39·1	0·08	0·02	0·04	43·7	0·14
Rhea	881	39·3	0·14	0·03	—	43·6	0·08
	883*	39·2	0·13	0·03	—	43·3	0·13
	1326	39·0	0·20	0·03	0·05	43·5	0·09
	1327	39·1	0·20	0·03	0·05	43·6	0·08
	1328	39·2	0·18	0·03	0·04	43·7	0·10
Mean		39·2	0·17	0·03	0·05	43·5	0·10
Ostrich	873	39·1	0·11	0·03	—	43·6	0·05
	874	39·1	0·14	0·04	—	43·8	0·09
	875	38·8	0·13	0·04	—	43·6	0·07
	876	39·1	0·14	0·05	—	43·7	0·07
	877	39·0	0·16	0·03	—	43·8	0·06
	878	39·0	0·15	0·03	—	43·8	0·05
	879	39·1	0·15	0·02	—	43·8	0·05
	880	39·2	0·14	0·03	—	43·7	0·06
	1207	38·8	0·16	0·03	0·06	43·4	0·07
	1298	39·3	0·12	0·03	0·06	43·4	0·04
	1300	39·1	0·13	0·03	0·04	43·4	0·04
Mean		39·1	0·14	0·03	0·05	43·6	0·06
Kiwi	1272	38·6	0·16	0·04	—	43·3	0·08
	1329	38·9	0·11	0·02	0·06	42·6	0·40
	1330*	38·9	0·15	0·02	0·06	42·7	0·19
Mean		38·8	0·14	0·03	0·06	42·9	0·22
Tinamou	1336	39·2	0·23	0·03	0·09	43·9	0·25
	1337*	38·6	0·16	0·04	0·04	43·5	0·35
	1338	38·2	0·20	0·03	0·03	42·5	0·24
Mean		38·7	0·20	0·03	0·05	43·3	0·28
Aepyornis	1359	38·7	0·25	0·01	0·02	44·0	0·03
Moa	1201	39·2	0·16	0·02	0·03	42·8	0·06
	1202	38·2	0·09	0·02	0·05	41·9	0·31
	1203	38·9	0·10	—	—	42·9	0·10
	1204	39·1	0·14	0·02	0·04	42·8	0·10
	1205	38·8	0·10	0·02	0·04	42·5	0·16
	1206	39·0	0·11	—	—	42·7	0·12
Mean†		39·0	0·12	0·02	0·04	42·7	0·11

* Samples probably from incubated egg.

† Omitting badly eroded sample 1202.

TABLE 4

Membrane, total, soluble and insoluble nitrogen expressed as percentage of true shell. Membrane values also expressed as mg. per cm².

Family	Serial No.	% membrane	Membrane mg/cm²	True shell % total N	True shell % sol. N	True shell % insol. N	Ratio insol. N/ sol. N.
Emu	882	2·21	5·80	0·30	0·16	0·14	0·9
	884	1·56	4·13	0·34	0·19	0·15	0·8
	1208	1·63	4·18	0·33	0·19	0·14	0·7
	1333	2·55	5·82	0·33	0·20	0·13	0·7
	1334	2·00	4·86	0·32	0·18	0·14	0·8
	1335*	2·75	6·46	0·32	0·18	0·14	0·8
Mean		2·12	5·21	0·32	0·18	0·14	0·8
Cassowary	1273	3·06	7·62	0·42	0·26	0·16	0·6
	1331*	3·00	6·24	0·26	0·13	0·13	1·0
	1332	2·73	6·68	0·34	0·18	0·16	0·9
Mean		2·93	6·85	0·34	0·19	0·15	0·8
Rhea	881	2·41	4·89	0·27	0·09	0·18	2·0
	883*	1·95	4·57	0·28	0·10	0·18	1·8
	1326	3·00	6·34	0·27	0·09	0·18	2·0
	1327	2·94	6·32	0·27	0·09	0·18	2·0
	1328	3·45	6·85	0·27	0·09	0·18	2·0
Mean		2·75	5·79	0·27	0·09	0·18	2·0
Ostrich	873	1·81	7·54	0·23	0·05	0·18	3·6
	874	1·48	6·40	0·24	0·04	0·20	5·0
	875	1·55	7·02	0·23	0·06	0·17	2·8
	876	1·48	6·36	0·23	0·04	0·19	4·8
	877	1·48	5·91	0·26	0·04	0·22	5·5
	878	1·21	5·67	0·26	0·06	0·20	3·3
	879	1·35	6·64	0·24	0·04	0·20	5·0
	880	1·67	7·35	0·26	0·06	0·20	3·3
	1207	1·48	6·54	0·26	0·06	0·20	3·3
	1298	—	—	0·26	0·05	0·21	4·2
	1300	1·61	7·12	0·26	0·05	0·21	4·2
Mean		1·51	6·66	0·25	0·05	0·20	4·0
Kiwi	1272	5·45	6·24	0·34	0·16	0·18	1·1
	1329	—	—	0·23	0·10	0·13	1·3
	1330*	5·99	6·08	0·26	0·11	0·15	1·4
Mean		5·72	6·16	0·28	0·12	0·16	1·3
Tinamou	1336	9·02	5·20	0·34	0·19	0·15	0·8
	1337*	7·20	3·28	0·23	0·10	0·13	1·3
	1338	5·81	2·89	0·34	0·16	0·18	1·1
Mean		7·34	3·79	0·30	0·15	0·15	1·0
Aepyornis	1359	—	—	0·17	0·05	0·12	2·4
Moa†	1201	—	—	1·14	0·24	0·90	3·8
	1202	—	—	0·61	0·15	0·46	3·1
	1203	—	—	0·99	−0·01	1·00	—
	1204	—	—	1·02	0·02	1·00	50·0
	1205	—	—	0·61	0·06	0·55	9·2
	1206	—	—	0·86	0·17	0·69	4·1

* Samples probably from incubated egg.

† Mean not given because of great variations probably caused by erosion.

There are clearly much greater differences between the various membrane and nitrogenous values as will be seen from Table 4. The table shows that percentage membrane values are very variable, but these values are a reflection of the shell thickness as well as of the amount of membrane, thus the thin tinamou and kiwi shells have a high percentage membrane, while the thick ostrich shell has a low value. The weight per unit area of membrane, as pointed out by Tyler & Geake (1953b), is a reliable guide to the absolute thickness of the membrane and these values show that all these large eggs, i.e., kiwi, cassowary, emu, rhea and ostrich, have membranes varying on the average from 5·21 mg/cm² to 6·85 mg/cm². The variation between individuals of one family is so great, however, that the mean differences are of little significance. The tinamous show the largest variation in membrane thickness, but the considerable difference in size of these three eggs may account for it. Membrane values were not available for moa and *Aepyornis* shells.

Mean total nitrogen values for the true shell vary from 0·25 per cent for the ostrich to 0·34 per cent for the cassowary, but here the values between individuals are far more consistent. For example, there appears to be a definitely higher value for the emu and cassowary when compared with the rhea and ostrich. The single value for the *Aepyornis* is very low, but this may be a result of its being very old and it may at some time have suffered erosion or leaching. The values for insoluble and soluble nitrogen are also fairly consistent within families but there are marked differences for the soluble nitrogen values between families. The large differences for total and soluble nitrogen shown by tinamou 1337 compared with the other two, is probably caused by the erosion which this shell has suffered (see later). Of particular interest is the ratio of insol. N : sol. N. This ratio appears to set reasonably clear lines of demarcation between different families, the only two sets of eggs giving roughly the same range of values being the emu and cassowary which are the two ratites usually classed as closely related and put into the same order. The significance of this ratio will be considered later.

RESULTS : INDIVIDUAL RATITES

Emu

General appearance

The emu egg shell has a very uneven surface caused by relatively smooth rounded granulations. The granulations may be large or small, the larger ones possibly being made up of a number of smaller ones ; they are of varying thicknesses. Between these granulations, there are depressions with flat bases and, as will be indicated later, these flat areas may be taken as representing a true surface, covered in other places by the granulations. If the shell has many granulations, only a little of the true surface is showing and these areas are isolated and fairly small. Since the granulations are almost black in colour and shiny, and the flat areas are greyish and dull, the general appearance of such shells is black and usually shiny. Where a shell has fewer granulations, the flat areas are more extensive and ramify in various directions. Such shells do not look black, but dark grey, and are not so shiny.

Examination of radial sections of untreated shell shows four distinct layers. The outer layer of granulations is black or very dark brown throughout its whole thickness. This layer will, of course, be missing in parts of the section. Below this is a thin, almost white, layer with a fibrous, rather open, appearance. Next comes a thick layer coloured green and, finally, a white inner layer. The line of demarcation between these inner layers of green and white is not absolutely clear cut, but there is little doubt of their distinctive character because, in addition to the colour, the white inner layer appears to consist of vertical columns tightly packed together and, nearer the membrane, separating into discrete knob-like structures, firmly embedded in the membrane. These two inner layers comprise most of the shell.

The inner layer, of vertical columns ending in knob-like projections, is the mammillary layer and the green layer is the spongy layer. The thin fibrous layer is only present in emu and cassowary eggs as far as is known at present, and, for reasons to be explained later, it will be given the name of resistant layer. The variable layer of granulations will be called the granular layer. Incidentally, these granulations must not be confused with abnormal concretions on shells for these granulations on the emu shell are truly part of the normal shell.

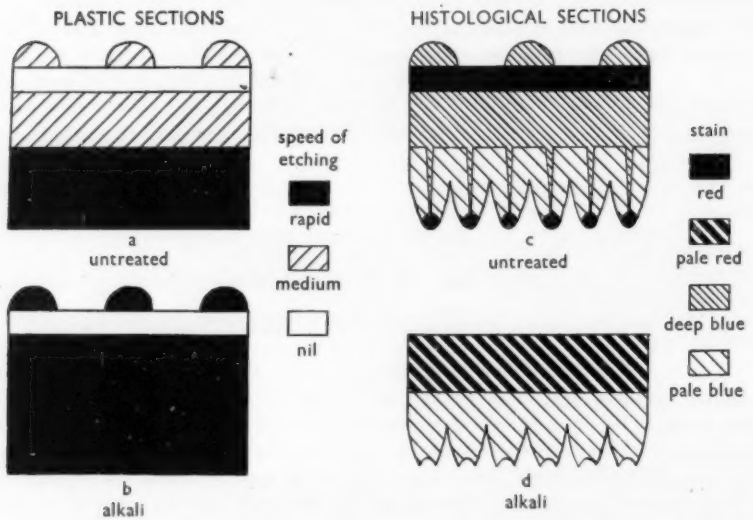

Fig. 2.—Emu shell. Conventional diagram showing (a) plastic-embedded, acid etched radial section of untreated shell, (b) plastic-embedded, acid etched radial section of shell previously treated with 10 per cent alkali, (c) radial section of untreated shell stained with Mallory's triple stain, and (d) radial section of shell previously treated with 10 per cent alkali stained with Mallory's triple stain. Only one projection from the spongy layer into each mammilla is shown. Membranes omitted from (c).

When a piece of emu shell is boiled in 2·5 per cent alkali, it loses its membranes and the clear-cut rounded mammillary knobs can then be easily seen. Parts of shell 1335 had already lost their membrane ; these pieces showed a hole in the end of each mammillary knob. This is an indication of erosion, or possibly, of incubation, since a similar characteristic has been noted in eroded moa shells (Tyler, 1957), and in incubated hen egg shells (Tyler, 1958). The granulations on the outer surface now look dull but have not altered in shape. The flat bottomed depressions are almost white in colour and many have holes in them and give the impression of an open structure underneath. Boiling with 10 per cent alkali changes the colour of the surface to pale grey and a fine white powdery deposit is found over all surfaces of the shell. However, if such a piece of shell is broken, the green spongy layer and the mammillary layer are still visible on the freshly broken radial surface.

Plastic embedded sections

Tyler (1956) has already described the structure of emu egg shells as revealed by the acid etching of plastic embedded shells. Briefly, the etching of a radial section gave four layers which are identical with the four layers already described. The granular layer is readily etched and the next layer, of fibrous structure, is quite resistant to etching, hence the name already given to it. The green spongy layer is fairly resistant and the mammillary layer is very readily etched (Fig. 2a).

When a shell is treated with 2·5 per cent alkali before embedding and etching, there is no difference in the etched picture, but when a shell has been treated with 10 per cent alkali, the etching result is quite different. This treatment makes no difference to the effect of etching on the resistant layer, but the spongy and the mammillary layers are now etched at equal speeds, namely, at about the same speed as that at which the mammillary layer was originally etched. It is then no longer possible to distinguish these two layers on the etched specimen (Fig. 2b). The granular layer also etches more quickly now.

The clear cut lines of demarcation on the etched specimens of untreated shell make it possible to measure the relative thicknesses of the various layers with considerable accuracy and the results, each the mean of four readings, are given in Table 5. The granular layer values are not given because, by its very nature, this layer is exceedingly variable, its thickness varying from 0 to about 17 μ in our specimens. From the mean values, it will be seen that the spongy and mammillary layers together make up about 85 per cent of the shell and that the spongy layer is usually the thicker, but, for individual shells, there are considerable variations.

Thin radial sections show dark projections from the spongy layer into each mammillary knob (Pl. 1, fig. 1), whilst, if tangential sections are cut, these projections then appear as dark triangles on a light background (Pl. 1, fig. 2). Near the mammillary core, the triangles are minute and increase in size if sections are taken higher up the mammilla.

When plastic mammillary casts are prepared (Tyler, 1956), it is seen that they consist of high or low walled cups and, in each cup, is a deposit (see Fig. 1b). This almost certainly represents the organic core present in the end of

TABLE 5

Emu and cassowary egg shells. Absolute (μ) and percentage values for the thickness of the resistant (R), spongy (S) and mammillary (M) layers. Etched radial sections of plastic embedded shells.

Serial No.	Absolute				Percentage		
	R	S	M	S/M	R	S	M
			Emu				
882	17	52	42	1·24	15	47	38
884	13	48	43	1·12	13	46	41
1208	12	40	45	0·89	12	41	47
1333	14	38	38	1·00	16	42	42
1334	15	46	39	1·18	15	46	39
1335*	16	46	34	1·35	17	48	35
Mean	14·5	45·0	40·2	1·12	14·7	45·0	40·3
			Cassowary				
1273	16	53	30	1·77	17	54	29
1331*	15	45	33	1·36	16	49	35
1332	18	49	38	1·29	17	46	37
Mean	16·3	49·0	33·6	1·46	16·6	49·7	33·7

* Samples probably from incubated egg.

each mammillary knob and will be described more fully later. These deposits usually exist as fairly flat discs but, in one case (1335), each deposit is so large that it fills its own cup completely. It is of interest to note that this sample is the one which has been either eroded or incubated. From the junction of a group of cups, spikes project. A proportion of these spikes continue as pore casts, fusing ultimately with the fibrous resistant layer. Thus the original pore channels run from the interstices between the mammillary knobs, up through the solid part of the mammillary layer, through the spongy layer, and terminate at the resistant layer as reported earlier by Tyler (1956). No pores go through this layer into the granular layer and this explains why no pore mouths are found on the egg surface.

A number of pores were removed from specimens of mammillary casts by breaking them off where they join the mammillary cups and then teasing the other end out from the resistant layer (Fig. 3). The pore casts are variable. At one extreme is the single pore, whose bore gradually widens towards the resistant layer. At the other extreme are pores which have either an ' arrow head ' shape or are forked to give two mouths. Usually the ' arrow head ' type may be considered as a forked type in which a connection between the two prongs of the fork has been maintained throughout their length and a suggestion of the forking may be apparent. In addition, there are compound pores made up of two separate pore structures fused together at the mouth.

In some samples a mixture of different types of pore is found, whilst in 1335, the pores are chiefly ' arrow headed ', but in 1334 they are almost entirely single and rounded. However, these selections only represent small areas of a shell and much more work is required about the variation of pore shapes over a whole shell.

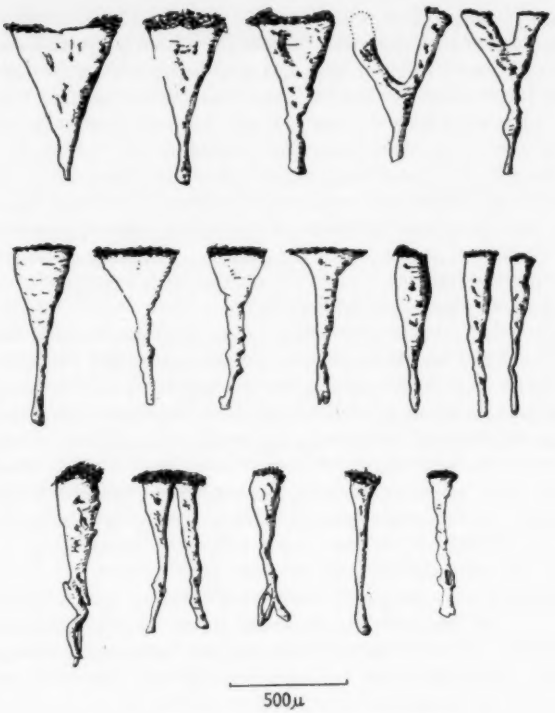

Fig. 3.—Plastic casts of emu egg shell pores.

Histological sections

When emu shells were decalcified using the method of Simkiss & Tyler (1957) and radial sections cut, the general structure as shown by Mallory's triple stain (Fig. 2c) was comparable in some respects with that obtained as described above, using the plastic embedding and etching technique. The granular layer on the surface stained an uneven purple colour, with small particles of red staining material in it. This layer rested on a continuous, rather openwork, layer containing large red staining granules embedded in blue staining connective material, the general effect being red. This is clearly the resistant layer of the etched plastic sections. These histological sections again show the resistant layer coming to the surface at some points and being covered by the granular layer at others. Beneath this resistant layer the rest of the shell takes up the stains in two distinct ways. Immediately next to the granular red staining layer, i.e., in the spongy layer, it is purplish blue, whilst it is a much paler blue in the mammillary layer. There is apparently no absolutely clear cut line of demarcation between spongy and mammillary layer, for the darker blue of the upper part dips as discrete projections into each mammilla

and these projections only terminate at the tip of each mammilla, which itself has a very small red staining core. These dark blue projections are thus seen against a background of lighter blue and may be equated with the dark projections of the thin radial sections of plastic embedded shell. The membrane stains red and is obviously fibrous. These fibres appear to pass through the red staining core, thus anchoring the membrane to the shell. Tangential sections through the mammillary layer show the dark blue projections as triangular in cross section and surrounded by the lighter blue material. They are large at the upper end and become smaller as they approach the red core of the mammillary knob, but, in any case, are relatively widely separated from each other. Again these agree with the dark triangles of the thin tangential sections of plastic embedded shell.

Treatment with 2·5 per cent alkali prior to decalcification results in the disappearance of the membrane, whilst 10 per cent alkali removes the protein core from each mammilla and also the resistant layer. The material in the mammillary layer continues to stain blue but the projections can no longer be seen. On the other hand, the spongy layer stains red, thus there is now a clear line of demarcation between these two layers (Fig. 2d). It should be noted at this point that, whereas the 10 per cent alkali treatment eliminates the line of demarcation between spongy and mammillary layer in the etched plastic embedded radial sections, it introduces a line of demarcation in histological sections stained with Mallory's triple stain.

Pore channels were seen in a number of sections and it was evident that they ceased at the red staining resistant layer, thus supporting the picture described earlier. There is evidence to suggest that some of the pore channels are well filled with material, but the majority are empty or are possessed of only a lining which stains faintly red.

Staining with iron haematoxylin gave the granular layer a purplish black, and the resistant layer a black colour. Below this the intensity of staining was greater in the spongy than in the mammillary layer. The projections into the mammilla stained weakly but could be distinguished, because the rest of the mammilla was almost unstained. The mammillary cores and the membrane were black. This staining picture was duplicated in bright orange when the tetrazo–β–naphthol method was used and thus it would appear that the resistant layer, the mammillary cores and the membrane are rich in protein, whilst the spongy layer contains less and the mammillary layer less still. In the mammillary layer, apart from the cores, it is confined to the projections from the spongy layer.

The PFAS test showed a positive result for the membrane and the resistant layer, indicating either disulphide groups as in proteins like keratin or double bonds between carbon atoms as in unsaturated fats.

The acetylated sudan black B test showed that a few pores were blocked with a material which gave a strong positive stain for fat. Fat was also noted in isolated patches in parts of the resistant layer. The granular, spongy and mammillary layers were only very weakly stained, but the mammillary cores stained strongly.

A section of shell was incubated with pepsin and it was found that only

the membrane and the resistant layer remained undigested. Repeating the PFAS test on this section showed that both these portions continued to give a positive result.

The ferri-ferricyanide and ammoniacal silver reactions were both positive for the granular layer, indicating the presence of reducing compounds. The resistant layer and membranes also stained. After treatment with acetic anhydride in pyridine, the tests were negative, thus showing that the reducing groups were now blocked. Further treatment, however, with potassium cyanide after acetic anhydride resulted in the development of more reducing groups, since the ferri-ferricyanide test when applied was again positive for the membranes and resistant layer. This suggests that the membranes and resistant layers are similar in that they contain disulphide groups as in keratin and confirms the PFAS test.

A positive result with the PAS test for all parts of the shell suggests the presence of polysaccharide material everywhere. However, the mammillary layer in general and the core and projections in particular, stained much more intensely. On the other hand, mucicarmine stained the mammillary layer, especially the material surrounding the projections, but not the cores, neither did it stain the remainder of the shell.

The use of mucicarmine and aniline blue together on a tangential section showed that the projections, here, of course, as triangular cross-sections, stained blue and the surrounding material stained red.

The material of the mammillary layer which surrounds the projections contains little protein as shown by tetrazo-β-naphthol test, but stains strongly with mucicarmine, indicating acid mucopolysaccharide. On the other hand, the projections from the spongy layer gave the strongest PAS reaction and continued staining blue with Mallory's triple stain even after the 10 per cent aklali treatment. This evidence suggests that the projections from the spongy into the mammillary layer contain a different protein—acid mucopolysaccharide complex from that in the spongy layer and that the material surrounding the mammillary projections is acid mucopolysaccharide without protein. Further, the PAS results indicate that the protein-acid mucopolysaccharide material in the projections from the spongy layer is richer in unsubstituted carbohydrate groups than that in the spongy layer. The unsubstituted carbohydrate material might also be more resistant to attack by 10 per cent alkali and might thus explain the results after this treatment.

Cassowary

General appearance

The cassowary egg shell is, in general structure, very much like the emu shell, in that it has a granular, resistant, spongy and mammillary layer. The granular layer is not so pronounced as in the emu, in that the deposits are neither so thick nor so extensive ; this latter state means that the spaces between the granulations are usually more extensive but they retain their flat-bottomed characteristics. The granulations are green, whilst the spaces between are pale yellow.

Radial sections show all the layers but they are by no means so clearly

distinguished as in the emu. This is particularly true of the spongy layer because its colouring is very much paler than in the emu.

Boiling the shell in 2·5 per cent alkali removes the membranes and reveals clear-cut rounded mammillary knobs. Sample 1331 appeared to be either eroded or incubated. The flat-bottomed spaces are now much paler in colour and, as in the emu, the surface is broken in places revealing an open structure beneath. Treatment with 10 per cent alkali again produced a pale grey shell covered on all surfaces with a fine white powdery deposit.

Plastic embedded sections

The cassowary egg shell gives exactly the same results as the emu shell in all aspects of the study, except for the actual measurement of the layers ; a separate set of diagrams has, therefore, not been drawn. The values for the layers as given by etching are shown in Table 5 and clearly do not differ greatly from the emu, but it should be noted that the means of the ratio of the thicknesses of the spongy and mammillary layers are different in the two sets of eggs. This situation would need to be investigated over a much larger

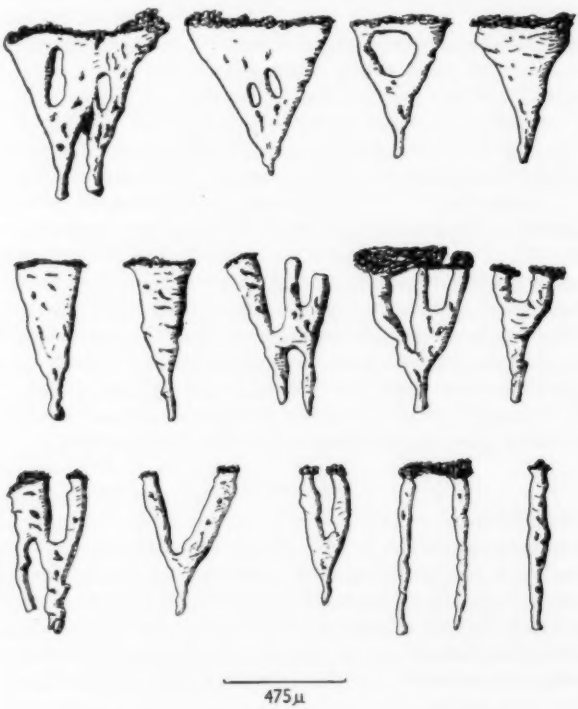

Fig. 4.—Plastic casts of cassowary egg shell pores.

number of eggs, however, before it could be considered in detail. The granular layer is, on the average, very much thinner in the cassowary.

Thin radial and tangential sections gave a similar picture to that for the emu.

The plastic mammillary casts also gave a similar picture to those of the emu, and, once again, the eroded or incubated sample (1331) gave cups filled with a white deposit. The pore casts gave single, almost cylindrical structures and also forked pores and ' arrow head ' pores of the type found in the emu. But there were further forms. Pores were found with three forks and also with two starting points, whilst, in one case, the ' arrow head ' filled the full length of the pore and left no stalk (Fig. 4). However, like the emu pores, they all ended at the resistant layer. It is of interest to note that Nathusius (1869) reported pore channels in the inner layers of the cassowary shell but could not observe the pore mouths because of the granular outer surface.

Histological sections

Once more the picture of the normal shell was almost exactly the same as in the emu and only the differences will be mentioned. The mammillary layer, as already noted in the plastic sections, tends to be thinner, but the smaller mammillae are seen to have somewhat larger cores. The only other difference is that the projections from the spongy layer are thicker and more extensive, giving rise to more protein and fat in the cassowary mammillae than in the emu. This also means that the areas of free acid mucopolysaccharide between the projections are proportionately smaller. The pores of these shells were completely devoid of a filling of organic matter.

Treatment with 2.5 per cent and 10 per cent sodium hydroxide solution gave exactly the same results as with the emu shell.

Rhea

General appearance

There may be two distinct kinds of shell in our specimens, because most are pale cream shells but two are green ; both kinds are shiny. It may be that these represent samples of *Rhea americana* and *Rhea darwini* respectively, but it must be borne in mind that *R. darwini* fades to a pale cream colour with age. Unfortunately, no information on the history of the eggs was available, but, in any case, the two kinds of shell showed no differences apart from colour. All the shells have pore mouth depressions, but, in addition, some have dimples in no way related to pore mouths. Most of the samples have deposits of dirt in the dimples and in the pore depressions. Some radial sections of untreated shell show two layers, the spongy and the mammillary layer, and it was possible to take rough measurements of these, but the line of demarcation between the two was not always very clear. The layers could be distinguished by the vertical structure of the mammillary columns and the amorphous nature of the spongy layer. In other shells the line of demarcation was impossible to judge but the two layers were present. In one shell there were indications of an extra very thin outer layer with a crystalline structure. Table 6 gives the results for the three shells where measurements were possible, each value being the mean of sixty to ninety readings.

TABLE 6

Rhea egg shells: absolute (μ) and percentage values for the cuticular (C), spongy (S) and mammillary (M) layers as seen in untreated shell and in etched radial sections of plastic embedded shells.

Serial No.	Absolute				Percentage		
	C	S	M	S/M	C	S	M
			Untreated shells				
1326	—	62	30	—	—	67	33
1327	—	63	31	—	—	65	35
1328	—	58	30	—	—	66	34
			Etched shells				
881	6	47	36	1·31	7	53	40
883*	8	67	34	1·97	7	62	31
1326	8	55	29	1·90	9	59	32
1327	7	54	37	1·46	7	55	38
1328	10	49	30	1·63	11	55	34
Mean	7·8	54·4	33·2	1·64	8·2	56·8	35·0

* Samples probably from incubated egg.

Note: The cuticular layer was not easily visible on the untreated shells and hence is included in the spongy layer. On the etched shells it was readily seen.

Treatment of the shells with 2.5 per cent alkali removed the membranes and also cleaned up the shells considerably, especially was it noticed that the dirt and debris was removed from the dimples and the pore depressions. It was now possible to see very clearly that the dimples which occurred in some shells bore no relationship to the pore depressions. The pore depressions contained one, two, and, very occasionally, three pore mouths, and with more than one pore mouth, the depression was elongated. These depressions were all orientated in one direction, except in a few pieces in which they appeared randomly arranged. The mammillae were now visible, being clear cut and rounded. In the green coloured shells, the inside of the shell was also green.

When 10 per cent alkali was used to treat the shells, they appeared to be covered on all surfaces with the same white powdery deposit which had been found on the emu shell similarly treated.

Plastic embedded sections

The results for all shells were the same in so far as three layers were revealed on etching as already shown by Tyler (1956). A thin outer layer, to be called the ' cuticular layer ', a spongy layer and a mammillary layer, Fig. 5a. It should be stressed here that this cuticular layer in the rhea is the thin layer of shell immediately beneath the cuticle ; it is not the cuticle itself. Again the layers were so clearly demarcated (Pl. 2, fig. 1) that measurements could easily be made and these are given in Table 6. There is considerable variability in the absolute thicknesses of the layers, between samples, but on a percentage basis this is somewhat reduced. The mean ratio of spongy to mammillary layer was greater than in the cassowary or emu. A comparison of these values for etched shells with those for untreated sections in samples 1326,1327

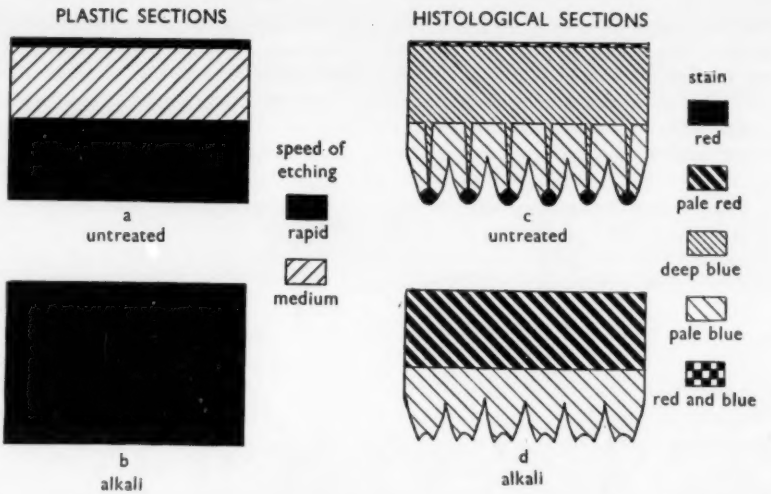

Fig. 5.—Rhea shell. Conventional diagram showing (a) plastic-embedded, acid etched radial section of untreated shell, (b) plastic-embedded, acid etched radial section of shell previously treated with 10 per cent alkali, (c) radial section of untreated shell stained with Mallory's triple stain, and (d) radial section of shell previously treated with 10 per cent alkali stained with Mallory's triple stain. Only one projection from the spongy layer into each mammilla is shown. Membranes omitted from (c).

and 1328, shows that, considering the roughness of the measurements on the ordinary shell, the visible mammillary layer of the ordinary shell corresponds with the rapidly etched layer of the plastic embedded shell and the spongy layer corresponds with the slowly etched layer of the plastic embedded section, plus the very thin rapidly etched cuticular layer. A faint indication of a cuticular outer layer was visible on only one sample of untreated shell.

On treating the shells with 10 per cent alkali before embedding, the difference in etching between the spongy and mammillary layers disappared, both now being rapidly etched (Fig. 5b, and Pl. 2, fig. 2). The outer cuticular layer, however, was occasionally still faintly visible as an even more rapidly etched portion.

A thin radial section showed dark projections from the spongy layer into each mammilla and the tangential sections revealed these same projections in triangular cross-section.

Mammillary casts gave clearly developed cups but in different shells the cups had varying heights of wall, in some cases groups of cups had a higher wall. There was the typical deposit at the base of each cup.

The pore casts were readily dissected off and showed the typical single or double mouths ; no triple mouth cast has yet been made, but this does not, of course, infer that they do not exist (Fig. 6). Nathusius (1869) reported that two pore mouths opened into a single longitudinal groove. In our samples the casts generally arose from an original one-pore channel and no example

was found of a two-mouthed pore depression resulting from two adjacent single pores. The proportion of singles to doubles was variable between samples but pore casts were too few in number to give any quantitative assessment of these data. In a number of cases it will be seen that the pore was multi-branched at the inner end, indicating that channels, each starting from the space between a number of mammillae, had coalesced.

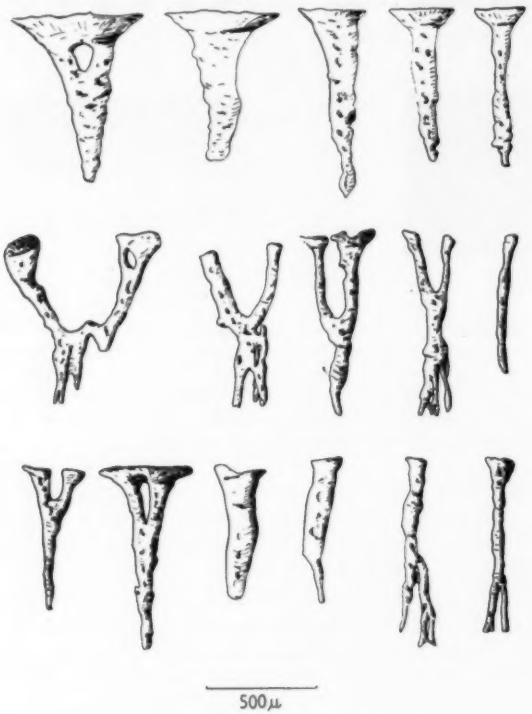

Fig. 6.—Plastic casts of rhea egg shell pores.

Histological sections

Mallory's triple stain shows, in radial section, a distinct cuticle which stains pale blue with small red staining areas at intervals. Over the pore mouths, the cuticle forms a thicker layer, staining almost entirely red.

Immediately beneath the cuticle is a thin region divided by many vertical gaps to give a crenellated appearance, possibly the cuticular layer of the etched specimen. Below this, the stain is dark blue in the spongy, and blue in the mammillary, layer. There are projections of spongy layer material into the mammillae and the red staining mammillary cores are small (Fig. 5c).

Shells treated with 2·5 per cent alkali before decalcification showed, as with

the emu and cassowary, the loss of membrane but no other marked change. However, 10 per cent alkali removed the cuticle and dissolved the red staining cores from the mammillary knobs and also changed the spongy layer from a blue to a red staining layer, but it had no effect on the general body of the mammillary layer, although the projections were no longer visible (Fig. 5d).

Sudan black B shows increasing amounts of fat in the outer layers and ammoniacal silver and ferri-ferricyanide give a similar gradient. The mammillary cores also stained strongly with Sudan black B. Iron haematoxylin gives increased staining in the outer portion of the shell, i.e., the spongy layer, whilst the tetrazo-β-naphthol reaction for protein indicates that there are large amounts in the spongy and mammillary layers but little in the cuticle, except over the pores.

The PAS and mucicarmine tests indicate that both spongy and mammillary layers contain acid mucopolysaccharide but the reaction is much stronger in the inner part of the shell. The PFAS test is positive for the membrane and negative for the rest of the shell unless a section contains a pore, then the plug of the pore gives a positive reaction indicating that it is a protein containing a disulphide linkage.

Ostrich

General appearance

The ostrich shells were either dirty grey brown, with debris in the pore depressions, or they were off-white to buff in colour. The former group were probably merely dirty through age, since boiling in 2·5 per cent sodium hydroxide solution cleaned them, more or less, to the buff colour. The clean shells had easily visible pore depressions and these were very variable in size and distribution.

Treatment with 2·5 per cent alkali made it possible to see pore depressions clearly in all shells and some shells had large numbers of large pore depressions into which the multiple pore mouths opened. Others had a mixture of large and small pore depressions, the small ones apparently having only one or two pore mouths, and finally some shells had few and small pore depressions, all apparently with single or double pore mouths. This treatment also removed the membrane while the mammillae in all samples showed up as clearly separated, rounded projections.

Shells treated with 10 per cent alkali suffered no apparent change, except that all surfaces of each piece were covered with the usual fine white powder.

In the above samples, treated and otherwise, it was possible to see the spongy and mammillary layers in a radial section, but the line of demarcation was quite indistinct and it was not possible to make any measurements.

Plastic embedded sections

The etched radial sections gave similar results in all samples, namely, a rapidly etched mammillary layer, a slowly etched spongy layer and, in some samples, the faintest suspicion of a more rapidly etched thin outer layer. No figure has, however, been drawn as the structure is fairly similar to the rhea.

TABLE 7

Ostrich egg shells: absolute (μ) and percentage values of spongy (S) and mammillary (M) layers as seen in etched shells.

Serial No.	Absolute			Percentage	
	S	M	S/M	S	M
873	113	68	1·66	62	38
874	100	90	1·11	53	47
875	116	92	1·26	56	44
876	102	84	1·21	55	45
877	101	75	1·35	57	43
878	106	96	1·10	53	47
879	100	114	0·88	47	53
880	102	91	1·12	53	47
1207	108	92	1·17	54	46
1298	122	69	1·77	65	35
1300	126	71	1·77	64	36
Mean	109	86	1·27	56	44

Measurements for the two chief layers are given in Table 7. There is a great deal of variation even when the values are expressed as percentages and values for the ratio of spongy to mammillary layer are also variable. It is, however, of interest to note that the two eggs laid by one bird at Ilfracombe Zoo show an S/M ratio much higher than all others except number 873.

Treatment with 2·5 per cent alkali did not alter this picture of two major layers, but when samples were treated with 10 per cent alkali before embedding and etching, the distinction between the two layers completely disappeared and both were now rapidly etched, a result similar to that for the rhea, emu and cassowary.

Thin radial and tangential sections once more gave strong evidence of projections from the spongy layer into the mammillary layer.

Plastic casts of the mammillary surface and the pores were also made. The mammillary surface casts were all very much alike, showing walled cups corresponding with the original well-separated mammillary knobs. Some of these walls were low but some were very high, especially those surrounding groups of cups. In some cases the walls were so low in a group of cups that it looked as if one large cup had two, three or even four deposits in it. From the meeting point of three or four of these cup walls, there arose individual branches of the pore casts, but some of these joined together later. These were dissected off and are shown in Fig. 7. The ostrich shell pores have always been described as multi-branched ever since Nathusius (1868) reported on them, but examination of samples from each shell available showed considerable variation. In some shells, the pore casts showed an exceedingly complicated system of channels, and one had so many tightly packed branches that the spaces between could not be cleaned out. In others the channels were much simpler but still multi-branched, whilst in other shells the system was exceedingly simple with a preponderance of single and double channels. The double channels are quite unlike the rhea forked pores where the single pore channels split into two

fairly near the surface. In the ostrich there were two distinct channels almost from the point of origin in the mammillary spaces and, in fact, the complicated systems seem to have many starting points. In a very general way the size and number of pore depressions observed on the surface of the original shell gave some indication of the simplicity or complexity of the pore system.

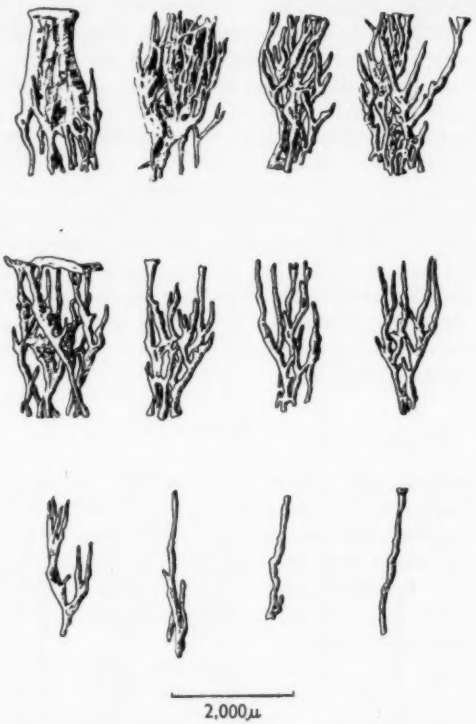

Fig. 7.—Plastic casts of ostrich egg shell pores.

Histological sections

Mallory's triple stain showed the general structure of the shell. In some shells the cuticle is poorly developed if one can be said to be present at all, for it is certainly poorly demarcated from the rest of the shell. The outer surface merely looks somewhat more compact with vertical cracks in it and a few red enclosures, otherwise it is slightly more deeply stained but the stain is blue like the rest of the spongy and mammillary layer. However, in other shells the cuticle is quite distinct and stains bright orange. The spongy layer consists of coarse fibres running parallel to the surface and if the section is opened out somewhat, the fibres appear to stick together in bundles with

individual fibres changing at intervals from one bundle to another, but this may be merely an artefact. The mammillary layer stains a paler blue but, again, the ostrich shell shows that projections of the spongy layer dip into each mammilla and reach right down to the tip of the mammillary knob, where there is a small red-staining core. The fibres of the membrane pass through this core.

In shells previously treated with 10 per cent alkali, the Mallory stain gives a distinct and clear cut line of demarcation between the mammillary and spongy layer, for now the former stains an overall blue while the spongy layer stains red. After this treatment the mammillary cores and membrane have gone completely.

The PFAS test was negative whilst Sudan black B stained the outer part of the shell and the mammillary cores. Tetrazo-β-naphthol, iron-haematoxylin, ammoniacal silver nitrate and ferri-ferricyanide, as well as the acid fuchsin in Mallory's triple stain, all showed more staining in the spongy than in the mammillary layer. With these stains, the membrane and cores and projections are also well stained. The PAS and mucicarmine tests gave less staining for the spongy than for the mammillary layer, whilst the projections, but not the membrane, showed very strong staining. It would thus appear as if there is a greater amount of protein and fat and a smaller amount of acid mucopolysaccharide in the spongy layer.

Kiwi

General appearance

The shells available were all similar in appearance, being dirty but smooth and having distinct pore mouths which appeared to be filled to the surface, not with debris but with organic material. Treatment with 2·5 per cent alkali cleaned the shells which were now white. The pore mouths lost a little of their filling by this treatment and, as far as could be judged, they were all single and most were of a very distinct funnel shape, the funnel at the surface varying in size from one to another.

In one shell the mammillae were intact but present as clumps rather than individuals, in another shell (1330) some mammillae had the normal rounded ends whilst others had a hole in the end. The third shell showed little sign of mammillae and was almost as smooth on the inside as on the outside.

When the shells were treated with 10 per cent alkali, a white powder covered all surfaces but no apparent major structural alteration occurred.

Plastic embedded sections

It proved rather difficult to obtain etched radial sections of kiwi shells and impossible to make a series of measurements. Nevertheless, a typical general picture emerged. The outer third of the shell is rapidly etched and the inner two-thirds only very slowly, but there is a slight distinction in this inner layer in that its own inner half is etched rapidly in such a way as to leave broad vertical columns with gaps between. This suggests that the individual mammillae are thinned down with acid but have a central, more resistant core which almost fills the large mammillae (Fig. 8a).

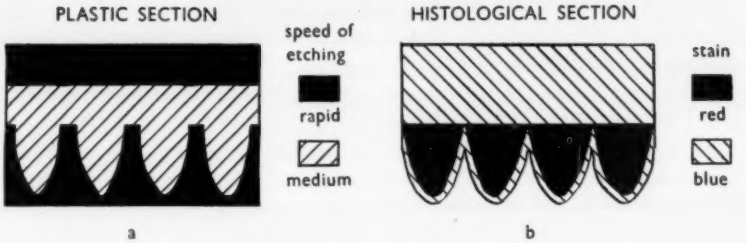

Fig. 8.—Kiwi shell. Conventional diagram showing (a) plastic-embedded, acid etched radial section of untreated shell, and (b) radial section of untreated shell stained with Mallory's triple stain. Membranes omitted from (b).

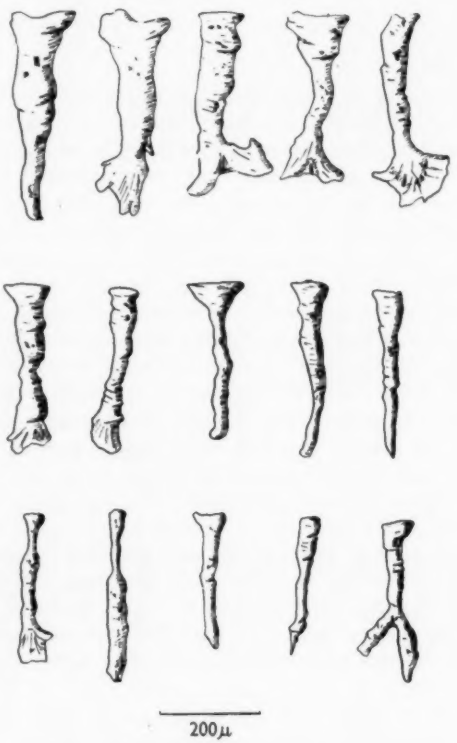

Fig. 9.—Plastic casts of kiwi egg shell pores.

Material from 10 per cent alkali treated shells, when embedded in plastic and etched, gave a completely broken and uneven picture in which no lines of demarcation could be traced.

Thin radial and tangential sections showed no signs of projections into the mammillae.

The mammillary cup walls can be seen in the plastic mould but it is impossible to judge their true height since they appear to be almost completely filled with deposit which, in some cases, even stands above the level of the surrounding walls.

Pore casts (Fig. 9) were all single-mouthed, the mouth sometimes being funnel shaped. In other pores they were relatively wide throughout their length. One cast was found with a forked channel at the inner end. It will be seen that a few of the casts have a " fish tail " at the inner end ; this is caused by the casts often breaking off much lower down than in other shells so that the " fish tail " represents part of the mammillary cup wall. Kiwi egg shell pores were reported by Nathusius (1871) as being small and sparse.

Histological sections

The structure of the kiwi shell as shown by Mallory's triple stain is simple (Fig. 8b). There is a cuticle which stains blue and is physically discrete from the spongy layer, but otherwise resembles it. The spongy layer forms about half of the thickness and stains blue, whilst the remainder is the mammillary layer. Each separate and large mammilla has only a little blue staining material round the outside, while the rest is almost entirely red-staining core.

Specimens treated with 10 per cent alkali then decalcified and stained with Mallory's triple stain, gave only very tattered, badly stained pieces of material. From the other families it is known that one of the actions of the 10 per cent alkali is to dissolve the mammillary cores and since, in the case of the kiwi, they make up such a large proportion of the total shell, it is perhaps not surprising that this treatment causes almost complete disintegration.

The PAS test was positive for the spongy layer and strongly so for the mammillae and the same is true for mucicarmine, except that the cores do not stain. Iron haematoxylin stained the membrane and mammillary cores black, but the spongy layer was unstained. This distribution corresponded to that of the protein as shown by the tetrazo-β-naphthol reaction which also indicated some protein in the spongy layer. Sudan black B gave results showing a great deal of fat in the mammillary cores and in patches of the cuticle where it appeared to be thicker, but with this shell, the mammillary and spongy layers also showed some fat. The ammoniacal silver and ferri-ferricyanide tests showed a similar distribution of reducing groups, mainly in the cuticle and cores.

Sections of pores which were obtained showed that they were relatively wide and funnel-shaped and seemed to be almost completely filled with material staining red with Mallory's triple stain. The PFAS test was positive, indicating disulphide groups and it may be that the material is denatured albumen.

Tinamou

General appearance

In the case of the three tinamou shells available, these were known to be from different species. One (1336) was greenish blue, 1337 was blue and 1338 was greenish ochre. All were shiny but 1338 was particularly highly polished. Apart from colour and some variation in membrane, shell thickness and shell nitrogen, the samples gave similar results for all the other tests. Pore mouths were clearly visible on the outer surface but they were filled flush to the surface with material which was probably organic matter. After boiling with 2·5 per cent alkali, the pore mouths were empty and could be seen to be funnel shaped. On the inner surface, the mammillae were visible but were not perhaps as clear cut as in the other shells of this study. Ten per cent alkali produced pieces which were covered with a white powder on all surfaces.

Plastic embedded sections

The tinamou radial etched sections were difficult to obtain but, generally speaking, the outer third of the section etched rapidly, the middle third very

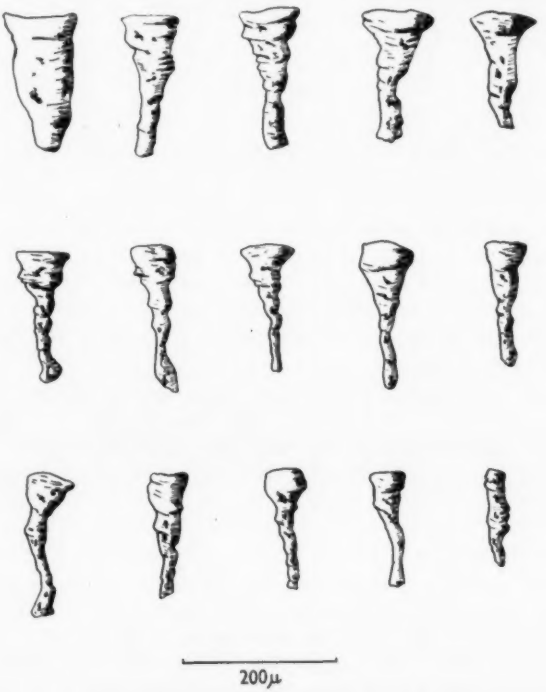

Fig. 10.—Plastic casts of tinamou egg shell pores.

little and the inner third was intermediate, but tended, like the kiwi, to give vertical etched spaces between less quickly etched columns.

Thin radial and tangential sections gave no indication of projections into the mammillae.

The mammillary surface gave very low cup walls with a relatively large deposit in them similar to those from the kiwi. The pore casts were approximately of the same type as found in the kiwi, except that none had ' fish tails ' (Fig. 10).

Histological sections

The tinamou shell seems to be very similar to the kiwi shell when stained with Mallory's triple stain. There is a poorly developed cuticle giving a slight stain with iron haematoxylin. The spongy layer staining indicates acid mucopolysaccharide with a little protein present, most of the protein is, however, found in the mammillary cores along with a great deal of fat. Ammoniacal silver and ferri-ferricyanide tests also suggest reducing groups in the mammillae.

The pores have the same general shape as in the kiwi and are full of a material which stains red with Mallory's triple stain. Further tests with iron haematoxylin, tetrazo-β-naphthol and PFAS suggest a protein containing disulphide groups.

Aepyornis

General appearance

Only two small pieces of *Aepyornis* shell were available. The outer four-fifths of the shell is pale in radial section and the rest darker brown. This darker brown layer may correspond with the mammillary layer for in it the structure appears to be vertical whilst the rest is amorphous.

On the outside of sample 1148 the pore depressions vary from single to double mouths with an occasional triple, quadruple or quintuple. All the multiple-mouthed slots are orientated in the same direction. On the inside there are only faint outlines of closely packed mammillae but there are clear indications of pore holes.

In sample 1359 similar pore mouth types are visible but, in addition, shallow channels have been cut in tortuous lines across the surface. On the inside surface, however, the ends of the mammillary knobs are much clearer.

In the case of these shells, it is highly probable that some erosion has taken place.

Plastic embedded sections

The radial section was equally rapidly etched at all levels and there was, therefore, no sign of any differential layers (Pl. 3, fig. 1). With sample 1148 the cast of the mammillary surface gave no sign of cups and hence the mammillae are too closely packed to give cup walls or else they have been eroded so much that their free ends have been completely lost. Sample 1359 gave quite good mammillary cup casts and these had a deposit in each.

Thin radial and tangential sections gave indications of projections into the mammillae.

Some excellent casts of pores were obtained (Fig. 11 and Pl. 3, fig. 1). These are relatively narrow in relation to their length, and, if having more than two mouths, they usually branch very near to the surface, but occasionally the

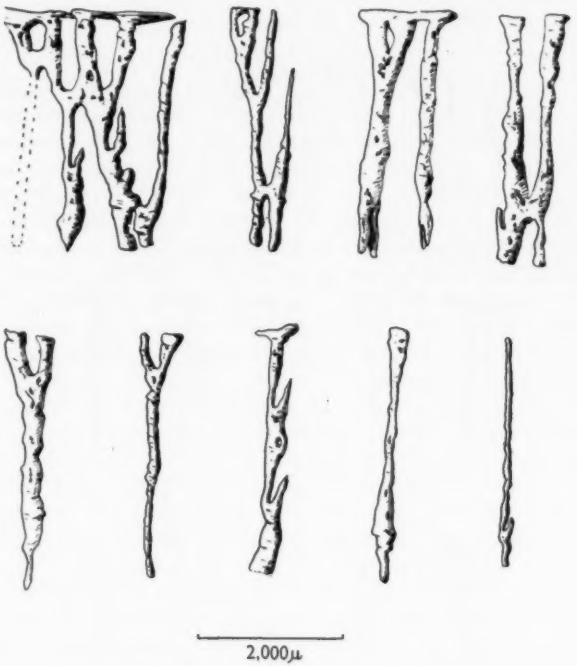

Fig. 11.—Plastic casts of Aepyornis egg shell pores.

two channels separate much lower down. With more complicated systems there is considerable joining up and separating of channels but this all occurs, unlike the ostrich pore channels, in one plane (Pl. 3, fig. 2). Nathusius (1871), reported that *Aepyornis* pores usually had two, sometimes more, mouths opening into sunken slots.

Histological sections

Sample 1358 was used to obtain decalcified material and, although it presented difficulties, a few sections were finally obtained. With Mallory's triple stain these showed a red-staining spongy layer and a blue-staining mammillary layer.

Moa

Tyler (1957) has given a full account of these shells elsewhere, but, briefly summarised, the position with regard to those samples which appeared relatively free from erosion is as follows.

General appearance

The samples showed typical elongated pore depressions into which one or more pore mouths opened, whilst the mammillary knobs were quite clear cut.

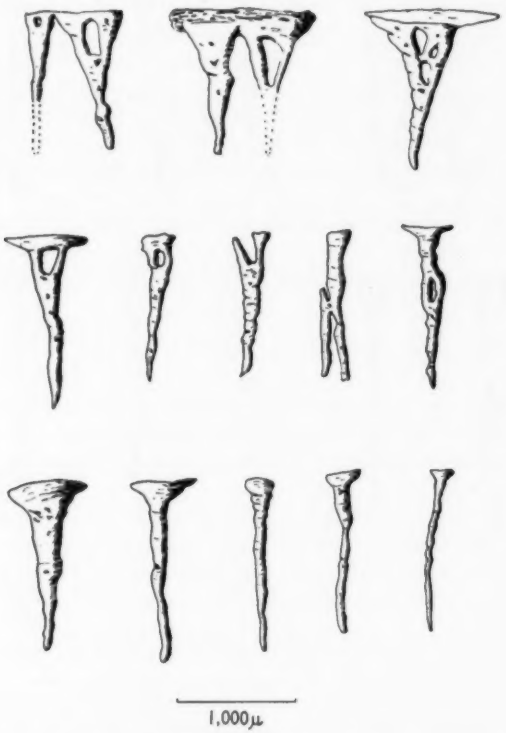

Fig. 12.—Plastic casts of moa egg shell pores.

Plastic embedded sections

The radial sections of uneroded shells were equally rapidly etched at all levels, whilst the mammilary casts gave cups with clearly defined walls and a deposit in each. Thin radial and tangential sections gave some faint evidence of projections into the mammillae.

A number of pore casts were obtained (Fig. 12) and these showed single and double mouths, whilst in addition, two pore systems opened into a common

pore depression on the surface, giving three or more mouths per depression. Nathusius (1870) had previously described moa pores as branched.

Histological sections

It was impossible to obtain any coherent organic structure after decalcification ; only a small quantity of debris was left.

DISCUSSION

General shell structure

The shells used in this study have been examined by general inspection, plastic embedding and etching techniques and by histological studies of decalcified sections. Almost without exception all the techniques led to the same general picture for any particular type of shell.

There seems to be little doubt that each ratite shell possesses as its major layers, well differentiated spongy and mammillary layers. The thin radial sections apparently show the presence of more organic matter in the spongy than in the mammillary layer. In all types, except kiwi and tinamou, there are projections from the spongy layer into the individual mammilla and in tangential sections these projections are clearly revealed as triangles surrounded by other material. Nathusius (1868) was the first to describe these projections which he saw in the ostrich shell. He stated that in thin tangential sections examined by transmitted light, the extreme end of each mammilla gave a dark pattern rather like the hub and spokes of a wheel, whilst sections cut at some distance from the end, showed dark triangles surrounded by transparent material. The further the section was from the end of the mammilla, the larger were the triangles. He considered that the triangles were cross sections of the vertical columns seen in radial sections. Nathusius suggested that the transparent areas consisted of a chemical combination of combustible material with calcium salts because a simple deposit of such salts would not be transparent. In succeeding papers, Nathusius reported these triangles in the rhea and cassowary (1869), moa (1870) and *Aepyornis* (1871), but, according to him, no triangles were present in the kiwi shell (1871). Schmidt (1957) has also described the triangles in the ostrich shell.

Etching techniques combined with alkali treatment show a distinct difference between spongy and mammillary layers and the histochemical studies confirm this in greater detail. These differences will be considered more fully in a later section.

Turning to the individual ratites, it is clear that the eggs of the emu and cassowary are very similar. The structure of the spongy and mammillary layer is the same, whilst they each possess a fibrous resistant layer above the spongy layer which has not yet been observed in any other egg shells. Above this there is a typical granular layer, again peculiar to the emu and cassowary. The resistant layer has been reported by Schmidt (1957), and according to him, Clevisch first mentioned it in 1913.

Casts of the pores show them to be fairly similar in type and in each case they terminate in the resistant layer.

The granular layer is calcified like the rest of the shell and its organic matter consists chiefly of protein and acid mucopolysaccharide whilst the resistant layer, also calcified, has a fibrous structure with its organic matter giving a positive test for disulphide linkages. The spongy layer is green in colour, fairly resistant to etching when impregnated with plastic and richer in protein than the mammillary layer below it. The mammillary layer is rapidly etched and contains, in general, less protein but more carbohydrate material than the spongy layer. What protein there is occurs in the projections and in the organic core of the mammillary knob. Fat is present in the outer part of the spongy layer and in the mammillary cores.

The rhea and the ostrich each possess well marked spongy and mammillary layers, which are readily distinguished by the tests applied. In the rhea there is also a clearly marked cuticular layer which shows up well in the thin plastic embedded radial sections and in the etched radial sections ; there is some indication of a similar layer in the ostrich shell, but the results are not so clear cut.

There is more protein and fat in the outer part of the shell than in the inner and the opposite is true of certain acid mucopolysaccharides. The mammillary knobs are relatively small, they may be arranged in groups and they show the typical organic core. The cuticle is poorly developed but each type seems to have vertical cracks in the surface of the shell which penetrate for a small distance.

Despite these similarities, the two types of shell show a major difference in their pore structure. The rhea has chiefly single or forked pores, but the ostrich pores may vary from the single type to pores of multiple origin, multiple branching and multiple mouths. This seems to distinguish the ostrich egg from all other eggs so far examined which, in addition to the eggs of other ratites, also includes the eggs of domestic hens, pea hens, guinea fowls, swans, geese, ducks, guillemots, razor bills, flamingos and penguins.

Nathusius (1870) maintained that the moa and rhea shells were more alike than any others. The moa shell has, therefore, been compared with the rhea and there is no doubt that the pore structure is somewhat similar. However, this is the only reliable evidence available, because the age of the samples of moa shell and the natural erosion and leaching to which they have probably been subjected, makes it difficult to draw conclusions from etched specimens and impossible to prepare decalcified specimens for histological work.

The *Aepyornis* has suffered similarly, but it was possible to obtain a histological section. The pore structure may perhaps be like the rhea but it runs in some cases to far more complicated systems. Histologically the structure is like a rhea or ostrich shell which has been treated with 10 per cent alkali, but there is little positive evidence relating it to either of these.

The kiwi and tinamou are very different from all the other shells in that their spongy and mammillary layers are not so obvious. Etching techniques gave some evidence of three layers, but treatment with 10 per cent alkali resulted in a blurred and useless etching which suggested a major breakdown. Histologically the shells have a poorly developed cuticle and, when compared with other shells, a thin, indefinite spongy layer and a very large well-developed

mammillary layer with big knobs almost full of core and little else. The protein and fat are concentrated in this layer. With such large cores which are known to be removed by the 10 per cent alkali treatment, it is not surprising that disintegration follows this treatment.

The pores in the kiwi and tinamou are simple in type and relatively wide compared with pores from much thicker shells. These pores are almost filled with some protein material which may be either keratin or denatured albumen.

Detailed shell structure

Simkiss & Tyler (1958) have recently suggested that the decalcified shell residue can act as a chelating agent. In their paper they measured the concentrations of various metal ions which were required to eliminate the metachromasia in a section of eggshell matrix. These measurements were called the " elimination concentrations " for the various cations and they were found to be related to certain constants of chelation. There was some indication in their experiments that the mammillary layer of the shell had a different " elimination concentration " for any particular cation, than had the rest of the shell matrix. This observation was elaborated in the present study by using cupric ions to eliminate the metachromasia. As the ratite eggshells are frequently thick, it was possible to study the different regions of the matrix in some detail.

TABLE 8

" Elimination concentrations " for the cupric ion/toluidine blue reaction, showing differences in different parts of the shell.

	Emu		Cassowary		Rhea		Ostrich		Kiwi		Tinamou	
Cu^{++} m.eq/l	1	2	1	2	1	2	1	2	1	2	1	2
100	0	0	0	0	0	0	0	0	0		0	
20	0	Tr	0	+	0	Tr	0	Tr	Tr		Tr	
10	0	Tr	0	+	0	Tr	0	Tr	Tr		Tr	
5	0	++	Tr	+	0	+	0	+	+		+	
2	+	+++	+	++	+	++	+	++	+++		+++	
0	++++	++++	++++	++++	++++	++++	++++	++++	++++		++++	

Column 1. Spongy layer.
" 2. Mammillary layer. 0 to ++++ = degree of metachromasia.

The results are shown in Table 8. From these it is clear that for all shells, other than kiwi and tinamou, the organic residue of the spongy layer has a much lower " elimination concentration " than has the material in the mammillary layer. This indicates that the chelating power of the spongy layer is greater than for the mammillary layer. Furthermore, the mammillary layer invariably gave a stronger PAS reaction.

When shells of the emu, cassowary, ostrich and rhea had been pre-treated with 10 per cent alkali, it was found that the spongy layer stained red with Mallory's stain compared with blue in an untreated shell, whilst the mammillary layer staining was unchanged, being blue in both cases. In addition, mucicarmine staining was weak in the mammillary layer of untreated shells, with

the exception of the emu, but in the 10 per cent alkali shells, it became positive.

All these reactions point to the fact that there are two different protein-acid mucopolysaccharide complexes in the shell and it is of great interest to note that the line of demarcation is quite clear cut, despite the projections into the mammillary layer which at first sight might be regarded as extensions of the spongy layer matrix.

The one exception to this general picture was the strong mucicarmine stain in the mammillae of the untreated emu shell. This is probably caused by the fact that there are very large spaces between the projections in this shell and it is these which stain with mucicarmine, whereas, in the other shells, the projections almost completely fill the mammillae and there are few spaces between them, hence there is only a weak staining with mucicarmine.

To account for this difference between the two protein-acid mucopolysaccharide complexes, it is suggested that there are two acid mucopolysaccharides in the egg shell, which will be referred to as AMPS I and AMPS II. AMPS I linked to protein occurs in the spongy layer : it chelates well (elim.conc. about 5 m. eq/litre Cu^{++}) and the complex with protein stains blue with Mallory's stain, but after pre-treatment of the shell with 10 per cent alkali, it stains red. It shows a weak PAS reaction and is mucicarmine negative. AMPS II occurs bound with protein in the mammillary layer, but its elimination concentration is high (20 m. eq/litre Cu^{++}) and hence it chelates less well. However, it shows a strong PAS reaction and it is stable to 10 per cent alkali as shown by an unchanged staining result with Mallory's triple stain. The alkali pre-treatment changes the mucicarmine stain from weak to strong. It would, therefore, seem as if the alkali treatment removes the protein from this complex in the mammillary layer and thus leaves behind the free AMPS II which now stains with mucicarmine. This free AMPS II also probably exists naturally in the spaces between the projections in the emu mammillary layer and would account for its exceptional positive mucicarmine staining in the untreated shell.

The strong PAS reaction for AMPS II suggests that it may contain proportionately more neutral sugars and less uronic acid residues in the chain than is the case with AMPS I and this would account for both its weaker chelating properties and perhaps its greater stability to alkali.

It is of interest to note that Tyler & Geake (1953a) showed that treatment with 10 per cent alkali under standard conditions removed a measurable proportion of nitrogen compounds from hen egg shells and Table 4 of the present paper shows the results for the various ratites. The analytical figures cannot, however, be readily equated with the changes in staining effects because the alkali has two entirely different effects, namely, that it completely removes the cores from the mammillary knobs and also removes protein from the spongy layer. In addition, it dissolves the resistant layer in the emu and cassowary shell. These two (or three) effects are not yet separable chemically. Nevertheless, there appear to be distinct family differences in the ratio of insoluble to soluble nitrogen. The ostrich and rhea give low soluble nitrogen values and these species have small mammillary cores and no protein

rich resistant layer. The emu and cassowary have small cores but a protein rich resistant layer and they show high values for soluble nitrogen ; on the other hand, the high values for kiwi and tinamou probably relate to the soluble nitrogen arising from the mammillary cores and possibly from blocked pores.

The radial etched sections, except those of kiwi and tinamou, are also of considerable interest in the light of the above results. Ignoring the granular and resistant layers in the emu and the cuticular layer in the rhea, there remains in all types of shell, a rapidly etched layer corresponding with the mammillary layer and a slowly etched layer corresponding with the spongy layer. This difference of etching again indicates two distinct layers and the difference may be caused by the two different protein-acid mucopolysaccharide complexes proposed. Further, after pre-treatment with 10 per cent alkali, the distinction between these two layers disappears for then both etch at rapid rates. It would seem that somehow the polymerised plastic can form a partial protection against the attack of acid in the spongy layer of the untreated shell, but that this capacity is lost when the shell is pre-treated with 10 per cent alkali. On the other hand, no protection occurs in the mammillary layer whether the shell is treated or not. It is not yet possible to say how this protective mechanism works, but it may be that the plastic monomer can co-polymerise with some groups in the spongy layer of the untreated shell, such groups being lost on pre-treatment with alkali, and never existing in the mammillary layer. Once more, there is a pointer towards the concept of different chemical structure and possibly of different acid mucopolysaccharides.

The complete resistance of the resistant layer in the emu and cassowary shells suggests that, in the untreated shell, the organic material is present in relatively large amounts and may be readily impregnated with plastic. Hence, although acid attack may dissolve calcium carbonate from between the plastic protein matrix, this merely gives an open network which does not diminish in general area or thickness. Similarly, when treated with 10 per cent alkali, so much of this organic matter is dissolved that the plastic has very free access to the whole area along large channels and the result is again a resistant layer.

At the other extreme is the thin cuticular layer in the rhea. This seems to be quantitatively so devoid of organic matter that no plastic can penetrate it, hence acid attack proceeds very rapidly indeed.

The kiwi and tinamou shells have not yet been discussed because they are quite different from the other four. The mammillary knobs are almost filled with the core which stains red with Mallory's triple stain, whilst what little other material there is in the mammillae is homogeneous with the material of the spongy layer. The " elimination concentration " is high (20 m. eq/litre Cu^{++}) for all the shell, whilst pre-treatment with alkali is so destructive that no results have been obtained. The evidence available does not indicate, therefore, which protein-acid mucopolysaccharide complex might be present.

Formation of pores

Examination of the selection of pore shapes given in Figs. 3, 4, 6, 7, 9, 10, 11, 12 shows that there is, very roughly, a type of pore for each ratite or group of similar ratites. In each case the simplest structure is an unbranched

pore, but, for more complicated structures, the variations on this theme separate into different types. In kiwi and tinamou there are no complicated structures. In the rhea there are some pores with two forks and some which show two forks with a closure again at the mouth. These are, however, only different in so far as the former has two mouths opening into two separate depressions whereas the latter has two mouths opening into a common groove. Most rhea pores originate in a single channel but a few are multiple. The moa agrees fairly well with the rhea in this respect. Cassowary and emu show two and three forks and also some multiple origins but, in addition, there are definite branchings in some emu pore channels which soon coalesce to give an arrow head formation as in Fig. 3. The *Aepyornis* shows much more variety than any of the previously mentioned ones, but this is far surpassed by the ostrich which not only has multiple origins and multiple mouths, but also multiple branching and reuniting.

Such variations make it very difficult to consider how pores are formed but some suggestions are perhaps worthwhile. Tyler (1956) has already pointed out that in the early stages of shell formation, when the mammillary knobs alone are present, there will be a channel between each group of roughly rounded mammillae and that each channel is a potential pore. As the knobs coalesce and the spongy layer is gradually added, then some of these channels will be blocked whilst others will be kept open, probably by liquid still moving into the egg from the oviduct. The presence in the kiwi and tinamou of pores blocked with protein gives some evidence in support of this idea of liquid moving through the pore channels. Most of the channels will be blocked early by deposited shell material, but even when the shell is complete, a small proportion will remain open and these are the final and complete pore channels. Tyler (1955) showed with hen and some other eggshells that pores are not distributed at random over the shell but that the presence of one pore at a particular spot tends to reduce the chance of another near it, whilst two near one spot reduce even further the chance of a third appearing there until, finally, with six pores to the square millimetre, the chance of a seventh occurring is negligible. It may be that the passage of liquid through the shell has something to do with this factor of distribution because, if pressure is reduced at one point by allowing liquid through, there is less need for a second channel near that point, whilst if there is a second channel, then there is even less need of a third and so on.

The tinamou and kiwi pores so far studied show no branching, whilst unpublished results from the hen show only a very small proportion of branched pores. However, in the emu, cassowary, rhea, moa and *Aepyornis*, there is branching of the pore channels. This may arise by a partial, instead of a complete, blocking of a potential pore channel which may then leave two potential branches to the original channel instead of one. If one of these channels is later blocked, then a cul-de-sac will form, which will show as a spur in the plastic cast.

It is not possible at this stage to visualise the actual factors which could give rise to branching but it cannot be caused entirely by chance because there

is a certain general type of branching associated with each family. Furthermore, it would be particularly difficult to fit the multi-branched ostrich pores into the same scheme as the rest of the ratites on a purely random basis, while there must also be some factor responsible for the alignment of the mouths of multi-mouthed pores seen in the rhea, moa and *Aepyornis*.

This is as far as the problem can be taken at the moment and to take it further will entail a huge task in making and drawing pore casts. Possibly a more extensive study of a number of shells all from one species or a complete study of one single shell, might further our knowledge.

Classification of the "ratites"

There can be little doubt that the ratites are not a primitive group, but have been derived from carinates (i.e. flying birds), and this suggests that they should not be considered as a fundamental split of the class Aves. Furthermore, the features upon which the classification systems are based have been shown to be largely due to neotenous features (de Beer, 1956). The situation has been put quite simply by this author, who says that whether the ratites represent a natural group, or whether they are an assemblage of forms which have independently followed parallel lines of evolution consequent on the loss of flight is a further problem for ornithologists to solve.

Turning now to the classification of these birds in relation to egg shell characteristics, the chief data is given in Tables 9, 10 and 11.

TABLE 9

General data for use in comparing the different ratite shells. Chemical analysis. Mean percentage values.

	Inorganic constituents in N-free shell				Nitrogen in true shell			
Family	Ca	Mg	CO_2	P	Total	Sol.	Insol.	Insol/Sol.
Emu	39·4	0·07	43·6	0·11	0·32	0·18	0·14	0·8
Cassowary	39·1	0·08	43·7	0·14	0·34	0·19	0·15	0·8
Rhea	39·2	0·17	43·5	0·10	0·27	0·09	0·18	2·0
Ostrich	39·1	0·14	43·6	0·06	0·25	0·05	0·20	4·0
Kiwi	38·8	0·14	42·9	0·22	0·28	0·12	0·16	1·3
Tinamou	38·7	0·20	43·3	0·28	0·30	0·15	0·15	1·0

The chemical analysis of the inorganic matter does not offer much help. The magnesium values appear to differentiate the emu and cassowary from the others, whilst the phosphorus values differentiate the kiwi and tinamou from the rest. The various nitrogen values are also not greatly helpful but the ratio of insoluble : soluble nitrogen separates the ostrich markedly from the rhea and the rhea, in turn, from the others. Although the distinction is perhaps not so clear cut, it is important to notice that the emu and cassowary give identical values and that these are lower than the kiwi and tinamou.

Etched radial sections of the plastic-embedded shells very clearly put the emu and cassowary into one group, kiwi and tinamou less clearly into another and the ostrich and rhea are quite different from the other four. All the birds

TABLE 10

General data for use in comparing the different ratite shells.
Platic embedding results.

Family	Etched radial section No. of layers	Mammillary layer triangles	Mouths per pore
Emu	4	Yes	1 or 2
Cassowary	4	Yes	1 or 2
Rhea	3	Yes	1–3
Ostrich	2 (3 ?)	Yes	Multi-branched
Kiwi	2	No	1
Tinamou	2	No	1
Aepyornis	1	Yes	1–5
Moa	1 ?	Yes	1–4

except tinamou and kiwi gave the dark triangles in the tangential mammillary layer sections. The ostrich pores are multi-branched, all the others are forked to varying degrees except kiwi and tinamou which are simple.

TABLE 11

General data for use in comparing the different ratite shells.
Histological results.

Family	Pore filling	Resistant layer	Mammillary size	Granular layer
Emu	Little	Yes	Small	Yes
Cassowary	Little	Yes	Small	Yes
Rhea	No	No	Small	No
Ostrich	No	No	Small	No
Kiwi	Yes	No	Large	No
Tinamou	Yes	No	Large	No
Aepyornis	—	No	Small	No

Turning to the histological data, it will be seen that the kiwi and tinamou pores are blocked with material, whilst the rest are not, and again these two have large mammillary knobs in relation to general shell size, whilst the rest have small ones. Finally, the emu and cassowary are the only birds which possess a granular surface and a resistant layer.

It is very necessary to guard against the desire to consider every distinguishing feature as a means whereby animals may be biologically classified ; nevertheless, there are a number of features of the egg shell which seem to indicate that the emu and cassowary are closely related and this has the support of the present day classification of the ratites based on other criteria. The ostrich and rhea are different, especially in pore shape, and both differ considerably from the cassowary and emu.

It is of considerable interest to note that the kiwi and tinamou have similarities in the chemical composition of the shells, in the etched radial sections, absence of triangles in the mammillary layer, pore shape, pore filling and in relatively large mammillae.

The difficulty in considering these points is to decide how many of them are related to other variables which would not form a satisfactory basis for classification. An outstanding problem here is that the shell thickness may affect pore structure. Furthermore, many of the features may be adaptive. An interesting speculation can be made regarding the similarities of the tinamou and kiwi shells. If the rate of formation of the rhea egg shell, for example, is taken as an arbitrary standard, then it is possible to explain the formation of the kiwi and tinamou shells on the basis of a change in the rate of shell formation. If a change occurs so that the rate of the early stages of shell formation is increased relative to the rate of the later stages, then the following changes in the structure of a " normal " eggshell would occur. Firstly, the mammillary knobs would be increased in size ; secondly, the pores would become more funnel-shaped ; thirdly, the pores would become blocked with albumen due to incomplete plumping of the egg, and, fourthly, the shell would possibly be thinner. These features are, in fact, the main distinctive characteristics of the kiwi and tinamou shells and are the points that would be considered if a classification were based upon shell structure. It would be dangerous, therefore, to classify the kiwi and tinamou together, because of their eggshell structure, but the possibility that changes in the rate of formation have affected the eggshell is of interest, particularly since de Beer (1956) has suggested changes in the structure of the bird too.

It is not wise to draw conclusions about the *Aepyornis* or the moa, because both these types of shell have suffered from erosion whilst lying buried in the soil. However, the absence of a granular and resistant layer suggests no relationship with emu and cassowary, whilst pore shape suggests no relationship with the ostrich, but possibly may relate them to the rhea and to each other.

SUMMARY

Egg shells of emu, cassowary, rhea, ostrich, kiwi, tinamou, *Aepyornis* and moa have been examined, chiefly by histological and plastic-embedding techniques. Chemical analyses for inorganic constitutents, membrane nitrogen and true shell nitrogen, including soluble and insoluble fractions, have also been carried out.

Of the inorganic constituents only magnesium and phosphorus show major differences between families. The thickness of the membrane is far less variable than is the percentage membrane because the latter is so greatly influenced by the varying thickness of the shell. Shells from different families showed considerable variation in the ratio of insoluble to soluble nitrogen in the true shell.

General inspection, histological tests and plastic embedding methods gave, in so far as they could, results which were in close agreement and threw considerable light on the structure of these shells. It has been found that shells from different ratites have different structural layers and also that there are variations in pore shape and the degree of branching of the pores. There seems to be a close relationship between the emu and the cassowary shell and also between the kiwi and the tinamou, but apart from these there are no other obvious relationships. The emu-cassowary relationship is probably

of taxonomic value, but this cannot be said of the kiwi-tinamou relationship.

Histological studies indicate that there is both protein and acid mucopolysaccharide material in the shell matrix and that different layers of the same shell may have different ratios of these and also different acid mucopolysaccharides.

These findings are used as a basis for a discussion of the general and detailed structure of the shells and some tentative suggestions are put forward regarding pore formation. The significance of the results in relation to the classification of the ratites is finally considered.

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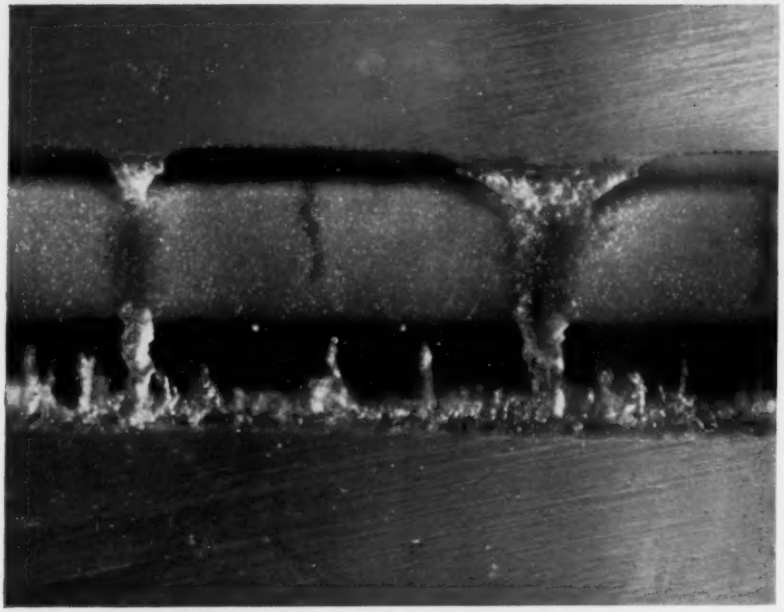
1

1. Emu shell. Untreated.

2

2. Ostrich shell. Untreated.



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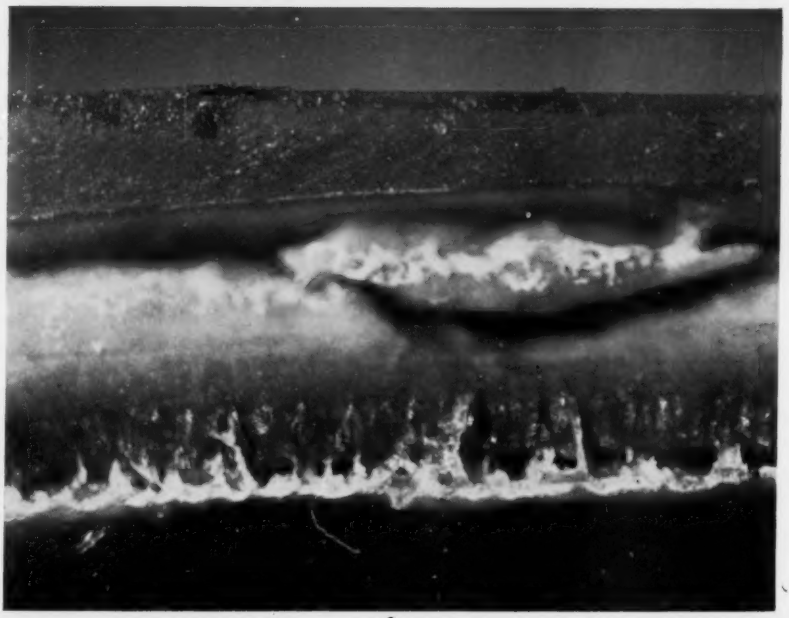

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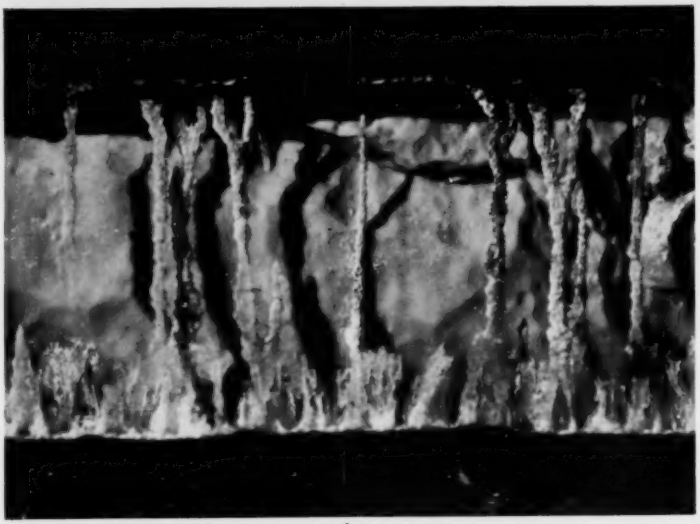
Rhea shell. 1. Untreated. 2. Treated with 10 per cent alkali.



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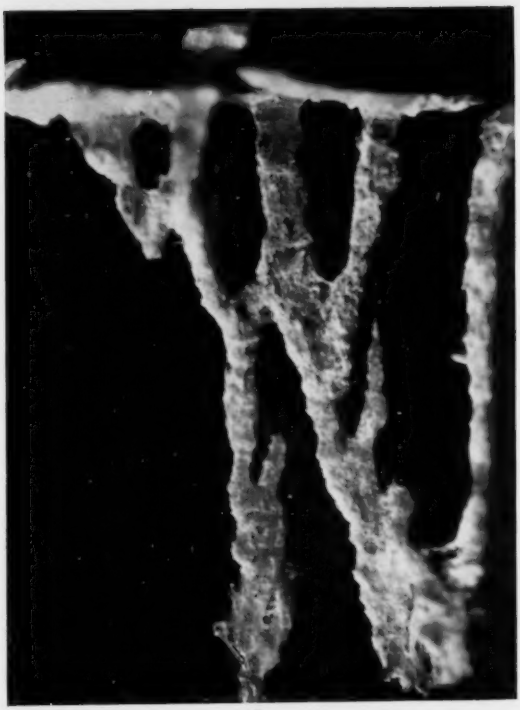

2

Aepyornis shell. 1. Untreated. 2. Plastic pore cast.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1.—Emu shell. Untreated. Thin radial section showing dark spongy layer and dark vertical columns in mammillary layer. Plastic-embedded section photographed using transmitted light. Shell thickness = 1150 μ.

„ 2.—Ostrich shell. Untreated. Thin tangential section through mammillary layer showing dark triangles surrounded by transparent material. Plastic-embedded section photographed using transmitted light. A similar picture was obtained for emu, cassowary, rhea, moa and Aepyornis, but not for kiwi and tinamou. (× 120).

PLATE 2

Fig. 1.—Rhea shell. Untreated. Plastic-embedded radial section, etched with acid to show differential etching of the different layers and also pore casts and walls of mammillary cups. The cuticular and mammillary layers etch more rapidly than the spongy layer. Photographed using reflected light. Shell thickness = 895μ.

„ 2.—Rhea shell. Treated with 10 per cent alkali. Plastic-embedded radial section etched with acid to show no differential etching between spongy and mammillary layer but a still very rapid etching of the cuticular layer. The extreme left of the picture shows the best result. Photographed using reflected light. Shell thickness = 895 μ.

PLATE 3

Fig. 1.—*Aepyornis* shell. Untreated. Plastic-embedded radial section, etched with acid to show no differential etching. Pore casts and walls of mammillary cups are clearly shown. Photographed using reflected light. Shell thickness = 3508 μ.

„ 2.—*Aepyornis* shell. Plastic pore cast. Photographed using reflected light. Total length = 3508 μ.

THE FEEDING HABITS OF THE WATER SHREW, *NEOMYS FODIENS BICOLOR* SHAW, IN CAPTIVITY AND THE EFFECT OF ITS ATTACK UPON ITS PREY

BY

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[Accepted 9th December 1958]

CONTENTS

	Page
Introduction	245
Observations	245
Discussion	248
Summary	249
References	249

INTRODUCTION

It has for long been known that the bite of the American Shrew *Blarina brevicauda* is poisonous. Seventy years ago C. J. Maynard (1889) reported feeling shooting pains up his arms after having been bitten by one and that less severe after effects lasted for more than a week. C. P. Pearson (1942) found mice and voles bitten by *Blarina* were seriously affected and that an extract of the submaxillary gland of *Blarina* could kill mice and rabbits when injected. He pointed out that the submaxillary glands of both *Blarina* and the European water shrew *Neomys* have a segment of coarsely granular cells which he considered to be the source of the poison in *Blarina*, and suggested the bite of *Neomys* might also be poisonous. More recently Harrison Matthews (1952) suggested any poisonous action might be of use to the shrew in overcoming large prey such as mice, but is more probably useful in minimising the struggles of large invertebrates, while M. Pucek (1957) found an extract of the submaxillary glands of the continental form of the water shrew, *Neomys fodiens fodiens* Schreber, when injected into the brains of mice and field voles produced convulsions, paralysis and death. When injected subcutaneously into mice, voles and frogs the extract produced no visible effect. He reported that the bite of a water shrew on a human being produced itching, a slight swelling and reddening of the skin. Pucek introduced live frogs and small mammals into the cages of the water shrews and found frogs only were attacked, and that though a frog bitten by a shrew jumped clumsily, there were no signs of paralysis. K. Z. Lorenz (1952) fed *Neomys f. fodiens* on tadpoles, small fish and, once, a frog. The last was attacked by several shrews at a time but seems only to have suffered the mechanical injury inflicted by the shrew's teeth. Of its aquatic prey he noted only "the shrew paddling shorewards with the wriggling creature in its mouth."

OBSERVATIONS

In September 1958 Dr Peter Jewell caught two water shrews and kindly gave one to me. He tells me that while handling the one he retained he was

bitten on the hand, in two cases to the extent of drawing small drops of blood. At the time he noticed that his fingers tingled slightly but was woken during the following night to find his hand smarting considerably where the shrew had bitten him : the discomfort had passed off by the morning.

Since it was difficult to collect its normal aquatic prey in adequate quantities, my own specimen was fed mainly on terrestrial invertebrates, and *inter alia* on earthworms, slugs and garden snails. The shrew obviously disliked the slimy secretion given out by these animals when attacked, on two occasions to the extent of giving a shrill, rather loud squeak, quite unlike the usual chattering, when its nose was covered with a sudden burst of frothy slime from a snail. Worms were regularly eaten and immediately attacked if offered, slugs and snails only for a day or two after capture and even then with no very great enthusiasm unless hungry. The observations which follow are the result of watching many attacks on worms, but few on slugs and snails. When attacking a worm the shrew invariably gave its victim a number of bites up and down the body, finally concentrating on the anterior end. This method of attack seemed to have a semi-paralysing effect ; following it even the largest worms finally lay more or less fully extended giving slow undulating writhes instead of the violent almost lash-like contortions made in the first stages of the attack. The shrew would then eat down the body, starting at the anterior end in every case observed. Dr Jewell tells me that his water shrew attacked worms in the same way, with the same result. On examination worms so attacked were found to have a number of constrictions down the body, apparently where bitten. The posterior portion, behind the last constriction, would often move strongly as if undamaged while a rather weak convulsive writhing movement took place between some of the damaged segments. The anterior portion more extensively bitten, was almost motionless. Even if removed from the dry peat moss litter on the floor of the cage and placed between layers of fresh damp moss such a worm was incapable of escaping. Large slugs seemed to be paralysed by a series of quick bites up and down the body, finally to lie still, only half contracted and no longer secreting any slime, but with slow waves of contraction passing up the foot. The shrew then started eating these too from the head. A large slug thus attacked and then left with part of the head eaten seemed narcotised to such an extent that when put into a 4 per cent solution of formalin it scarcely contracted at all and gave off no protective slime, in contrast to a live un-narcotised one put into the same solution. In the case of small snails with shells 5 to 7 mm. in diameter, the shrew quickly broke the shells, extracting and eating the snail. In one case, a snail with a shell some 10 mm. in diameter, the shrew attacked the snail at the foot without breaking the shell and after a minute or so pulled the entire snail out of the shell and ate it. Large snails up to 20 mm. in diameter were attacked at the foot, part of which was eaten ; in no case was the shrew seen to pull one of the large snails out of its shell but in every case the shell of the snail which had been attacked was found to be empty within a few hours if the shrew had been given no other food.

This method of attacking worms—" quick bites along its whole length "—by both moles and shrews has been recorded before by Barrett-Hamilton &

Hinton (1910–21), indeed it has been said that moles paralyse worms by biting the two ganglia in the head and can store them, as a badger is reputed to store " pithed " frogs, alive but incapable of escape, for future use. This is clearly a mistaken interpretation of the effect of the mole's bite, since " worms with these [supra and sub-oesophagal] ganglia removed are more sensitive than normal worms and therefore restless in natural conditions " (G. S. Carter, 1940). In the case of the shrew under review one large worm had the first half dozen segments removed, on another an attempt was made with a pin to stimulate the mechanical damage inflicted by the shrew's teeth about the body and head. Both these thereafter continued to move in a normal way and each in turn was given to the shrew which seized it, gave it a number of bites up and down the body and proceeded to eat it beginning at the anterior end as usual. Each worm after the shrew had eaten a segment or two was removed and found to be semi-paralysed. The paralysing effect does not seem necessarily to be lethal if conditions are favourable to recovery. If given a worm when it was not very hungry it would bite it up and down the body, eat a few segments and leave it. In such cases, left on dry peat moss litter the worm would lie almost motionless and, unless eaten in the meanwhile, die. It seems possible however that the dry surroundings prevent recovery. A large worm bitten and semi-paralysed, and with two or three of the anterior segments eaten, was removed and placed between two layers of fresh wet moss. After half an hour there did not seem to be any change in its condition and it still lay, twitching gently between the layers. Two hours later there was a weak wave of contraction and the worm had started to move into the moss. In another two hours it was in little better condition. It was then left over night and in the morning was much stronger, reacting more or less vigorously when picked up, though obviously not fully recovered.

The shrew had in its cage a small tank in which to swim and in which food in the way of worms, slugs, snails, etc. were put. On two occasions its normal food—small fish, 30–40 mm. in length, water snails, water shrimps, aquatic insects, etc. was placed in this tank. In every case, as Lorenz (1952) noted, the shrew carried its prey, even small insects, ashore to eat. Worms, slugs and snails put into the tank were also carried ashore. The fish it would carry ashore, bite up and down the body and start eating at the head end. Two ten-spined sticklebacks and one three-spined stickleback, taken from the shrew and returned to water within a few seconds of being caught and being bitten as described, were stiff and apparently paralysed, showing no signs of movement, the three-spined stickleback with the dorsal and ventral spines standing out stiffly. None of the above were damaged about the head but may have been damaged along the back bone. Two small loach similarly removed from the shrew and returned to the water showed no signs of paralysis and seemed as lively as ever. Usually all these small fish were quickly killed by bites into the head, but all were carried ashore alive.

A common shrew (*Sorex araneus*) when given a worm attacks it in a like manner to that used by a water shrew, it bites it and "eats down the body" in the same way. When dealing with a very large worm it will also sometimes give it a few more bites along the body but its attack does not seem to have any

paralysing effect. Even when a common shrew has eaten several segments from the head end down a worm will continue to writhe very actively. A large worm when attacked by a common shrew and with a number of the anterior segments eaten, when removed, was able to move forward in the normal way, a strong wave of contraction passing down its body. The common shrew will hold down the body of a small worm with its feet as it eats it and will try to do the same with a large one, but in the latter case the shrew is constantly thrown off its balance by the contortions of the worm, sometimes it almost seems as if it is being thrown from side to side ; there are certainly no signs of a paralysing effect upon the worm either by a poisonous secretion or by the mechanical action of the shrew's bite on the body of the worm or destruction of the ganglia at the anterior end by mastication. Medium sized and large slugs and snails were not attacked by a common shrew, though small snails up to 5 mm. in diameter were quickly broken and the snail eaten. Small slugs, less than 20 mm. in length when extended, were usually eaten so quickly that it was impossible to remove one in order to see if it were paralysed. In one case, however, the slug was removed with the anterior third of the body eaten : it was half extended and did not contract when picked up, but in 4 per cent formalin contracted immediately, though not completely, and gave off slime.

The discovery of "stores" of semi-paralysed worms in mole runs and nests is well authenticated and Harrison Matthews (1952) points out that the mole has a very large submaxillary gland the nature of whose secretion is unknown. A mole (*Talpa europaea*) kept in captivity usually attacked large worms with " quick bites along the body " as described by Barrett-Hamilton & Hinton (1910–1921) and then started eating from the anterior end. When eating the mole would hold down its victim with its fore feet pulling the anterior portion towards it between the feet while the posterior portion continued to writhe and contort violently even down to the last inch or so. In every case worms bitten up and down the body and with a few anterior segments eaten when taken away from the mole were able to move normally, a strong wave of contraction passing down the body. If given more worms than it could eat the mole would sometimes bite them, carry them to one place and bury them together, pushing earth over them with its nose. If the earth were damp and loose the lightly damaged worms would then move away, those more seriously damaged, e.g. with up to half eaten, would move aimlessly about with a more or less normal wave of contraction. There were no signs of paralysis. Slugs and snails were not attacked.

DISCUSSION

It is difficult to see how the more mechanical damage inflicted by the shrew's teeth can cause this paralysis. A slug under continued attack or irritation will give off slime, contract and remain contracted. Damage to the head alone will certainly not cause paralysis, a decapitated slug contracts violently in formalin. In the case of a snail the muscle by the contraction of which the animal retracts into its shell when attacked will not relax again if the attack continues, not even if the foot is pulled mechanically to such an extent that the animal is broken in two. It seems therefore that the bite of the water

shrew must in some other way paralyse the muscle, making it relax and so allowing the shrew to extract and eat the snail, just as it has been shown to paralyse the muscles in a slug. In earthworms paralysis does not follow the bite of a common shrew which attacks its victims in a manner similar to that used by the water shrew, not even in the case of small worms where the damage inflicted by the common shrew's smaller teeth would be comparable with that inflicted by a water shrew on a large one. Nor does paralysis follow the bite of a mole which also uses a similar method of attack and whose larger teeth would inflict much greater mechanical damage. That worms, given time and suitable conditions, seem to recover from the effect of a water shrew's bite suggests that the paralysis is due to some toxic effect and not to mere mechanical damage. It would seem there that the poison found by Pucek (1957) in the submaxillary gland of the water shrew must be the cause of the paralysis produced by that animal's attack upon its invertebrate prey.

The effect upon vertebrates is doubtful. Pucek (1957) and Jewell both noticed more discomfort following a bite than would be expected from the mere mechanical damage. Pucek found that a subcutaneous injection, a more effective method of introducing poison than a bite, had no obvious effect upon mice and frogs, while Lorenz (1952) observed nothing more than mechanical damage on a frog and that fish were carried ashore still wriggling. The three sticklebacks mentioned above may have been paralysed by damage to the spine and the loach were apparently not affected at all.

SUMMARY

A poison is known to be secreted by the submaxillary gland of the European Water Shrew but this does not affect small mammals or frogs when injected subcutaneously. The feeding habits of a captive water shrew are described and it is suggested that this poison, transmitted by its bite, enabled it to minimise the struggles of its larger invertebrate prey. The bites of a common shrew and of a mole have no such effect.

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THE ANATOMY IN RELATION TO FEEDING HABITS OF *FORFICULA AURICULARIA* L. AND OTHER DERMAPTERA

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(With 28 figures in the text)

CONTENTS

	Page
Introduction	251
Techniques	252
Body form and arrangement of sclerites	252
General structure of the head	254
Morphology of the mouthparts	257
General features	257
Labrum	257
Mandibles	258
Maxillae	259
Hypopharynx and cibarium	260
Labium	262
Mouthpart movements	262
Feeding	263
The cranial nervous system	264
The neck	269
Functional morphology of the neck	269
Cervical nerve supply	274
Action of haemocoelic fluid	275
Structure of the abdomen	276
The heads and necks of other Dermaptera	278
Discussion	278
General trends in the evolution of the dermapteran head	282
Dermapteran types	284
Brain size	296
Summary	297
Acknowledgments	298
References	299

INTRODUCTION

Food collecting is the most primitive and fundamental of all animal activities and for that reason a study of the feeding habits of any groups of animals gives an insight into its general biology. Amongst the mandibulate orders, there is a general superficial similarity in the form of the mouthparts, but there is also a wide range in structural detail and mode of action, which is associated with differences in feeding habit. This is an aspect of insect anatomy and morphology, which has hitherto been neglected and an account of the feeding habits of any of these orders would fill a gap in existing knowledge.

No order of mandibulate insects is more neglected than the Dermaptera, which has, therefore, been selected for study, *Forficula auricularia* L. being taken as a representative type because it is common and widespread and its natural history better known than that of most earwigs. Of the more important publications on dermapteran anatomy and morphology are Henson's (1946, 1950 and 1951) papers on the malpighian tubules and external morphology of the head and thorax of *Forficula auricularia*, Strenger's (1950 a) description of the head muscles of the same insect, Pantel's (1917) classical paper on the thorax of *Anisolabis* and Strenger's (1950 b) comparative study of the cercal muscles of a number of earwigs. These descriptive papers take little account of the function of the structures mentioned. Hudson's (1947) account of the dermapteran tentorium is inaccurate, while Crampton (1909–1932) and Martin (1918) failed to appreciate the specialised nature of the dermapteran neck. Apart from detailed descriptions of genitalia in taxonomic papers, the structure of the abdomen remains uninvestigated.

TECHNIQUES

Feeding was observed with the aid of a binocular microscope. The earwigs were viewed from various angles whilst feeding on stems, leaves and other matter. The movements of the mouthparts, head and abdomen were recorded and the type of damage noted.

External features have been drawn from living, dried and alcohol preserved specimens. Bouin or Carnoy fixed material has been dehydrated, placed in methyl benzoate for a week then in 1 per cent celloidin-methyl benzoate solution for a similar period, sectioned in ceresin wax at 10 μ and stained with Ehrlich's haemotoxylin and eosin or by Masson's technique. Haematoxylin stained celloidin sections were prepared in the usual way and used for graphical reconstructions.

The diagrams of the mouthparts have been drawn from projections of slides of whole mounts.

BODY FORM AND ARRANGEMENT OF SCLERITES

The common earwig is a semi-subterranean insect living in loose soil, litter, hollow plant stems, under bark and other similar situations. Here, according to Crumb, Eide & Bonn (1941), it feeds upon a wide variety of plant and animal matter with a strong preponderance of plant material. The insect is adapted to these situations by being dorso-ventrally flattened and in having the legs laterally attached to the thorax. In lateral view the wedge-shaped prognathous head fits closely into the front of the prothorax, which dorsally bears a large shield like pronotum. The thoracic segments are inclined forwards to accommodate the powerful coxal muscles, those of the prothorax being the smallest and the metathorax the largest. When not in use, the membranous hind wings are folded under the mesothoracic tegmina, which are held together in the mid-dorsal line by a series of inter-locking setae (Henson, 1951). The abdominal segments slant forwards, and laterally the terga and sterna alternate. The sclerites of one segment overlap those of the next two to four segments :

an arrangement which permits great abdominal flexibility and extensibility and provides a means of maintaining a haemocoelic fluid pressure.

The sclerotised unsegmented cerci are used as organs of offence, for perceiving the height of the ceiling in a crevice and can also be used to lever the insect backwards out of difficult situations. Many earwigs use the cerci to hold the prey during feeding, but this has not been observed in *Forficula auricularia*.

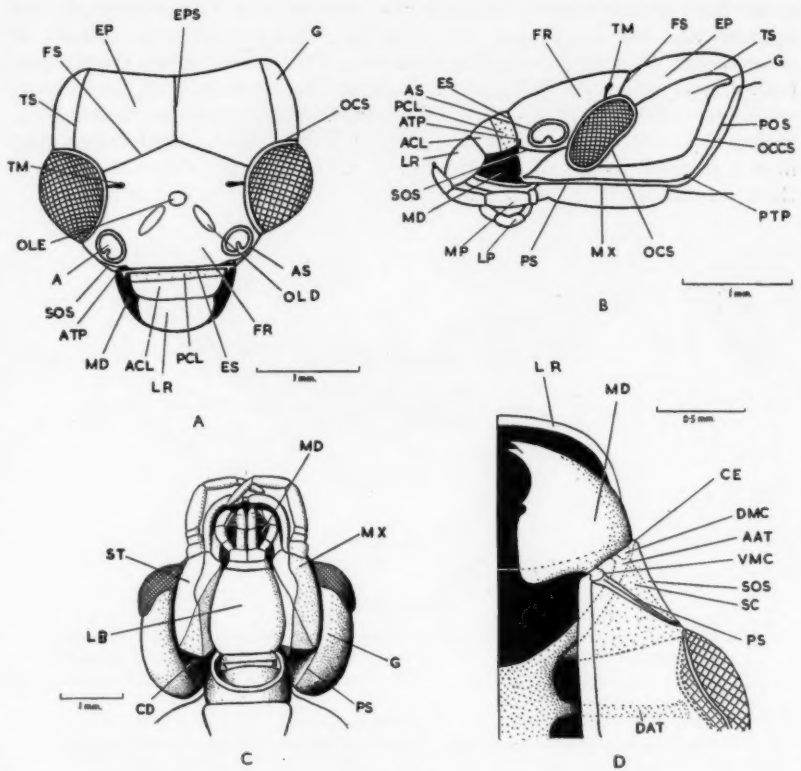

Fig. 1.—Head capsule of *Forficula auricularia* L. A. Dorsal view. B. Lateral view. C. Ventral view. D. Ventral view of left anterior region to show internal struts to the mandibular condyles.

A, antennal socket; AAT, anterior arm of tentorium; ACL, anterior clypeus; AS, antennal sclerite; ATP, anterior tentorial pits; CD, cardo; CE, cranial edge; DAT, dorsal arm of tentorium; DMC, dorsal mandibular condyle; EP, epicranium; EPS, epicranial suture; ES, epistomal suture; FR, frons; FS, frontal suture; G, gena; LB, labium; LP, labial palp; LR, labrum; MD, mandible; MP, maxillary palp; MX, maxilla; OCCS, occipital suture OCS, ocular or orbital suture; OLD, origins of labral depressor muscles; OLE, origins of labral elevator muscles; PCL, posterior clypeus; POS, post occipital suture; PS, pleurostomal suture; PTP, posterior tentorial pit; SC, sheet of cuticle between dorsal tentorial arm and cranial wall; SOS, Subocular suture; ST, stipes; TM, tentorial macula; TS, temporal suture; VMC, ventral mandibular condyle.

GENERAL STRUCTURE OF THE HEAD. (Fig. 1)

In many respects the head of *Forficula auricularia* resembles the generalised hypognathous type of insect head (as previously described by Snodgrass, 1928 and 1935) and with which the head of *Locusta* closely approximates. The head of *Forficula* differs from both types of head in having the ventral side between the mandibles and maxillae elongated (Henson 1950). This causes the region of the head capsule anterior to the frontal sutures to be rotated forwards and upwards and the mouthparts are, therefore, directed forwards instead of downwards. The elongation of this hypostomal region also effects the tentorial body and adjoining structures and is functionally associated with the rotatory movements of the maxillae during feeding. In addition the head has become prognathous by an elongation of the ventral side of the neck and the foramen magnum is inclined backwards. making an obtuse angle with the ventral side of the head, instead of a right angle as in *Locusta* (cf. Fig. 2A and 2C).

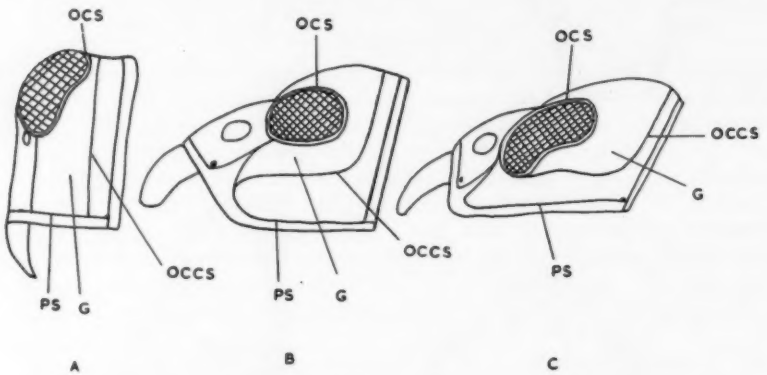

Fig. 2.—A comparison of the heads of the generalised insect and *Forficula auricularia*.
A. Lateral view of the generalised insect head, redrawn from Snodgrass (1935).
B. Lateral view of a hypothetical intermediate stage between the primitive insect head and that of *Forficula auricularia*.
C. Lateral view of the head of *Forficula auricularia*.
G, gena; OCCS, occipital suture; OCS, ocular suture; PS, pleurostomal suture.

Anteriorly the cranial edge (Fig. 1D, CE) is bent round the bases of the broad thin horizontally moving mandibles to form a grip and to provide articulating facets for the mandibular condyles (Fig. 1D, DMC, VMC). The genae (Fig. 1, G) are dilated laterally and posteriorly and the original ventral side of the head is carried inwards. The maxillae and labium, therefore, lie in a median longitudinal groove on the underside of the head between the inflated genae (Fig. 1C).

The mouthparts, in general, resemble those of the Orthoptera but there are some important adaptive differences, which will be described later.

In *Locusta* the eyes and antennae are situated near the dorsal region of the head. If these sense organs occupied similar positions in the prognathous

head of *Forficula*, the eyes would point upwards and backwards and the antennae upwards. In *Forficula* the compound eyes are situated nearer the mouth and the antennal sockets have migrated laterally anterior to the eyes.

Like the tentorium of *Locusta*, that of *Forficula* (Fig. 3) is composed of a central body joined to the inner wall of the cranium by two pairs of stays or arms. The anterior arms are formed by invaginations from a pair of pits

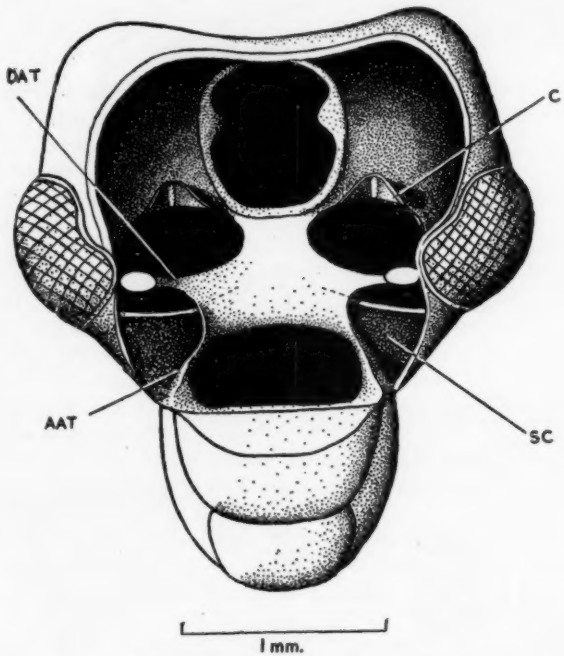

Fig. 3.—Dorsal view of the head capsule with part of the roof removed to show the tentorium, and the sheet of cuticle between the anterior arms of the tentorium and the pleurostomal suture. The condyles for the cardoes are shown one on either side of the ventral margin of the foramen magnum.

AAT, anterior arms of tentorium; C, condyle for maxillae; DAT, dorsal arm of tentorium; SC, sheet of cuticle.

situated laterally one on each side, in the epistomal suture (Fig. 1B, ES). Internally the anterior arms (Fig. 1D, AAT) are joined by a sheet of cuticle (Figs. 1D, 3, SC) to the subocular sutures (Fig. 1, SOS). The dorsal arms of the tentorium (Figs. 1D and 3, DAT) arise as branches of the anterior arms and extend upwards towards the cranial roof, to which they are attached near the posterior corners of the frons (Fig. 1, FR). This point of junction is indicated externally by a pair of shallow depressions known as the tentorial maculae (Fig. 1, TM). The body of the tentorium is elongated compared with that of *Locusta* and is,

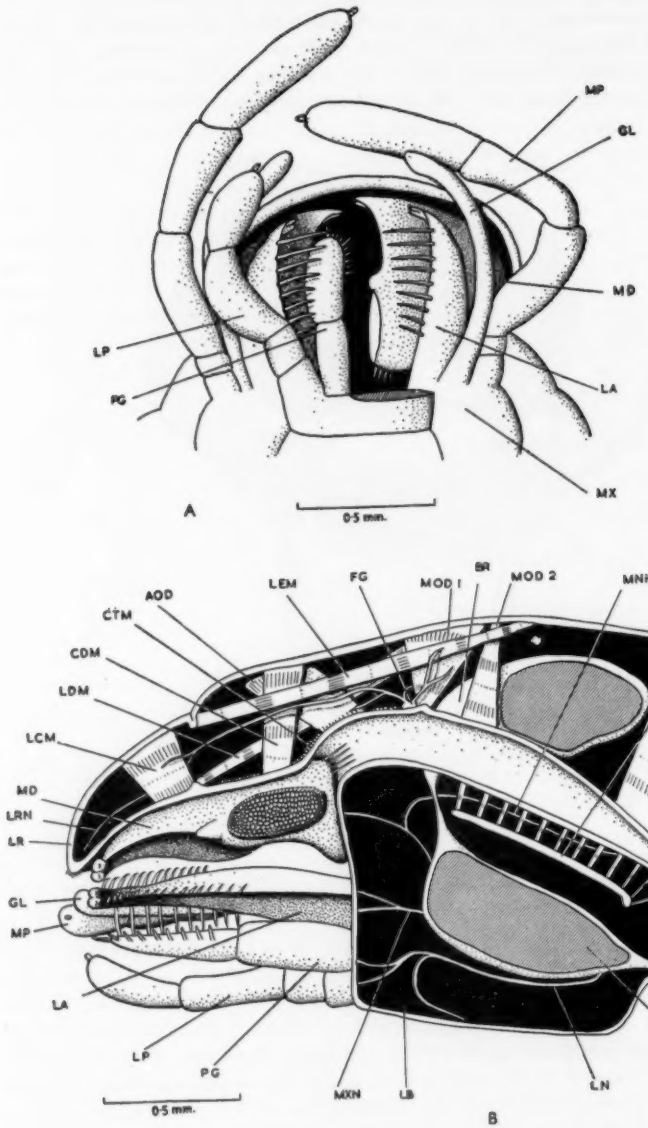
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Fig. 4.

as will be shown later, the origin of the large maxillary adductor muscles (Fig. 9, CAM, SA), which play an important part in the feeding habits of these earwigs. The posterior arms of the tentorium arise as small pits (Fig. 1 PTP), the posterior tentorial pits, situated in the post-occipital suture, one on either side of the head. The function of the tentorium is to form an internal brace to the head capsule and to act as the origin for a number of muscles concerned with the movement of the antennae, maxillae and labium.

MORPHOLOGY OF THE MOUTHPARTS. (Figs. 1C, 4 and 5)

General features

The mandibles and distal segments of the maxillae with the hypopharynx lie in a forwardly directed pouch at the front of the head, formed from the labrum, dorsally and the labium, ventrally. The cardoes and stipes of the maxillae lie lateral to the labium (Fig. 1C). The mouthparts are approximately horizontal and the maxillae and labium, therefore, form the median portion of the ventral wall of the head capsule.

Labrum

In dorsal view the labrum (Fig. 1, L) appears semi-circular in shape and is posteriorly attached to the clypeus along its straight hind margin. In longitudinal section (Fig. 4, L) it has a convex dorsal wall and a slightly concave ventral wall. The anterior edge of the labrum bears short orally directed setae which encourage the movement of food particles towards the mouth, but not in the opposite direction.

The proximal edge of the labrum is invaginated to form a loop of thick elastic cuticle and to which, on each side, are ventrally attached a pair of sclerotised rods, the tormae (Fig. 7, T)—one rod being directed forwards and the other backwards. The labrum can be raised and retracted by the contraction of paired median labral elevator muscles (Figs. 4B and 7, LEM) which originate posteriorly on the underside of the frons and are inserted dorsally on the hind wall of the labral loop described above. As the labrum is raised the anteroclypeus is displaced posteriorly and the flexible cuticle of the posterior clypeus (Fig. 1, PCL) is folded under the front end of the frons (Fig. 1, FR). In

Fig. 4.—Mouthparts and associated structures.

A. Ventral view of the mouth parts of *Forficula auricularia* to show the relative positions of the mandible, lacinia and paraglossa. The left paraglossa and labial palp have been removed.

B. Lateral view of a median longitudinal section through the head to show mouthparts, cibarial and oesophageal muscles and the cranial nervous system.

AOD, anterior oesophageal dilator muscles; BR, brain; CDM, cibarial dilator muscle; CTM, cibarial transverse (depressor) muscles; FES, junction of frontal and epicranial sutures; FR, frontal ganglion; GL, galea; LA, lacinia; LB, labium; LCM, labral compressor muscle; LDM, labral depressor muscle; LEM, labral elevator muscle; LN, labial nerve; LP, labial palp; LR, labrum; LRN, labral nerve; MD, mandible; MNN, mandibular nerve; MOD1, first median oesophageal dilator muscle; MOD2, second median oesophageal dilator muscle; MX, maxilla; MXN, maxilla nerve; MP, maxillary palp; PG, paraglossa; POD, posterior oesophageal dilator muscle; SOG, suboesophageal ganglion; TB, tentorial body with ventral oesophageal dilator muscles.

addition to these movements the labrum is depressed by the contraction of the labral depressor muscles (Figs. 4B and 7, LDM), which originate laterally on the frons and are inserted on the posterior tormal rods. The positions of the origins of these two pairs of muscles are indicated on the upper surface of the frons by two pairs of shallow depressions (Fig. 1, OLD, OLE). The labrum is also lowered by the contraction of the labral depressor muscles (Fig. 4B, LDM), which originate medially near the ventral labral apodeme and are inserted medially on the upper side of the labrum.

Mandibles (Figs. 1D, 4A and 4B, MD)

The mandibles are dorso-ventrally flattened and each has a slightly convex upper surface and a flatter ventral lower surface. Distally the mandibles are thin, but become thicker nearer the mouth. Distally each mandible bears two hard sclerotised medially directed teeth, one being slightly behind and below the other. The second tooth of each mandible is joined by a cutting edge to a third smaller tooth nearer the mouth. As the mandibles close the distal teeth on the right side come to lie slightly dorsal to those on the left and the two cutting edges impinge on each other like the blades of a pair of scissors, and when closed the right cutting edge is dorsal to the left and the third teeth are in contact. Immediately behind the third tooth of each mandible is a median oval molar area composed of stiff setae (cf. Fig. 4B). Near the posterior lateral corner of each mandible is a dorsal and a ventral condyle (Fig. 1D, DMC, VMC). Each condyle is spherical in form and is joined to the posterior wall of the mandible by a short posteriorly directed peduncle. The two condyles are attached to opposite sides of the mandible, one being dorsal and the other ventral. Each condyle fits into a cup-shaped depression or articulating facet, forming a ball and socket joint. The mandible therefore can be rotated only inwards or outwards in a horizontal plane. During mandibular closure the condyles tend to be forced outwards, but are held in position by internal struts (Fig. 1D). The dorsal condyles (DMC) are held in place by a sheet of cuticle (Figs. 1D and 3, SC) between the anterior tentorial arms (AAT) and the subocular suture (SOC) The ventral condyle (VMC) is supported by an internal thickening of the dorsal arm of the pleurostomal suture (PS). Comparison with the generalised head or with that of *Locusta* suggests that these modifications may have arisen as follows. By an enlargement of the cuticular fold of the epistomal suture (Fig. 1B, ES) between the anterior tentorial pits (ATP) and the subocular suture (SOS) an internal sheet of cuticle is formed between them. As the distance between the anterior tentorial arms and the subocular suture increases posteriorly, the extra length of cuticle seems to be derived from the antero-ventral corners of the genae, which, thereby, are invaginated into the subocular suture. In *Forficula* this invagination appears to be so large that the junction of the occipital and anterior pleurostomal sutures is also carried into the subocular suture. This causes the pleurostomal suture to become L shaped and the occipital sutures originate in the ocular suture. (Fig. 2).

On the posterior median corners of the mandible are a number of orally

directed setae (Figs. 4A, 4B and 5B) which assist food into the functional mouth during mandibular closure.

The mandibles are opened by a pair of abductor muscles (Fig. 8, ABM), which originate on the posterior ventral wall of the cranium and are inserted on the lateral posterior corners of the mandibles by apodemes which run, one on each side, ventral to the dorsal and anterior tentorial arms. Mandibular closure is due to the contraction of the large adductor muscles (ADM) which are inserted by a thick apodeme (one on each side) on the posterior mandibular wall medially and originate on the genae and epicranial sclerites. Behind the brain the apodemes become L shaped in cross section ; the bottom arm of the L pointing laterally. From the two arms of the L muscle fibres run respectively sideways and upwards to the cranial wall. On each side, the dorsal longitudinal tracheal trunk runs forwards from the neck between the two sets of muscle fibres.

Maxillae (Figs. 4A, 4B and 9)

The maxillae occur, one on each side, between the labium and the inflated gena (Fig. 1C) and in general are constructed on a similar plan to those of *Periplaneta*, which is frequently figured in standard entomological text-books. Each maxilla lies slightly under the lateral margin of labium to which it is connected by arthrodial membrane. This arthrodial membrane forms a narrow band round the base of the cardo, but expands anteriorly having a maximum width at the distal end of the stipes. The maxilla is laterally connected with the head capsule by a similar triangular band of arthrodial membrane, the two being joined anteriorly. As a result of these features haemocoelic fluid tends to push the maxillae forwards, downwards and outwards.

The cardo and stipes are divided into a number of subsidiary sclerites (see Henson, 1950) of little functional significance. The cardo-stipes joint can be bent through about 90 degrees and by this means the maxilla is swung forwards or backwards : the cardo and stipes moving as a pair of hinged plates. The short transverse vertical cardo (Fig. 1C, CD) articulates its median corner with the floor of the head capsule by a special condyle (Fig. 3C) upon which the maxilla can be also rotated outwards by contraction of the posterior promotor muscle (Fig. 9, PPM), which originates ventrally on the hind wall of the head capsule and is inserted on the ventral edge of the cardo by a special apodeme (see Fig. 9). The cardo is rotated forwards and medially by the contraction of two adductor muscles (Fig. 9, CAM), which originate latero-ventrally on the main tentorial body and then run obliquely downwards and outwards to be inserted ventrally on the stipes.

The stipes (Fig. 1C, ST) is a large rectangular sclerite, which posteriorly articulates with the ventral wall of the cardo. The maxillae are retracted by the contraction of the cranial lacinial flexor muscles (Fig. 9, CLF), which originate on the hind wall of the head capsule and are inserted on the inner side of the lacinial bases. Retraction of the maxillae is, therefore, accompanied by lacinial adduction—the functional significance of which will be discussed later. There are, however, no muscles capable of moving the maxillae outwards and

downwards and it seems that movements in this direction are due to haemocoelic fluid pressure. The experiments described on page 275 support this view.

The five-jointed maxillary palp calls for little comment. The palp is raised and lowered by special muscles inserted on the palp base and originating on the front of the mentum (Fig. 9, MPD and MPE). Figure 9 also shows the arrangement of the palp muscles, which, with the exception of those of the proximal and distal segments can only bend the palp inwards. Palp extension is, therefore, probably mainly due to haemocoelic fluid pressure.

The galea (Fig. 4, GL) is a cylindrical appendage, curved slightly inwards. At its base is a ring of hard elastic cuticle. The galea is hinged at the outer edge of the base and is bent inwards by the galeal flexor muscle (Fig. 9, GF) which originates ventrally on the stipes and is inserted medially on the base of the galea.

The lacinia is a long inwardly curved appendage, distally bearing a pair of medially directed teeth, situated immediately ventral to those of the mandibles. Near the apical teeth the inner surface of the lacinia faces medially and posteriorly, but towards the lacinial base comes to face more and more medially and upwards. The median edges of the laciniae bear a row of short dorso-medially directed setae with orally recurved tips (Fig. 4B). Along the ventral edge of the laciniae is a row of medially directed setae. When the laciniae are closed these dorsal setae lie just in front of the molar areas of the mandibles. The lacinia is hinged at the outer edge of its base which is thickened to form a ring of hard elastic cuticle. The lacinia is bent inwards by the contraction of the stipes lacinial flexor muscle (Fig. 9, SLF), which originates laterally on the ventral wall of the stipes and is inserted medially on the lacinial base. Lacinial flexure is also due to the cranial lacinial flexor muscle described above. In the absence of any special muscles for abduction of the galea and lacinia it would appear that these movements are performed either by the elasticity of the cuticular bases, haemocoelic fluid pressure or a combination of both processes.

Hypopharynx and cibarium

The hypopharynx (Fig. 5A and B) is a large fleshy lobe attached to the posterior wall of the cibarium, ventral to the oesophageal opening. The dorsal hypopharyngeal wall forms the floor of the cibarium and is composed of soft elastic cuticle strengthened by a number of V-shaped elastic chitinous rods (Fig. 5A). Two small lobes—the superlingulae (Fig. 5, SL) are attached, one on either side, laterally to the distal region of the hypopharynx, giving it a shovel like form when depressed. The anterior dorsal wall of the hypopharynx bears many small orally directed setae and these are particularly numerous immediately in front of the salivary cup (Fig. 5A, SLC) : a median pore into which open the ducts from the two pairs of salivary glands (Fig. 5B, DSG, VSG). Immediately posterior to the salivary cup there is a median longitudinal row of stiff molar setae, which are immediately ventral to a similar row of setae on the cibarial roof (Fig. 5B, CMS). Both sets of setae impinge on the molar setae of the closed mandibles.

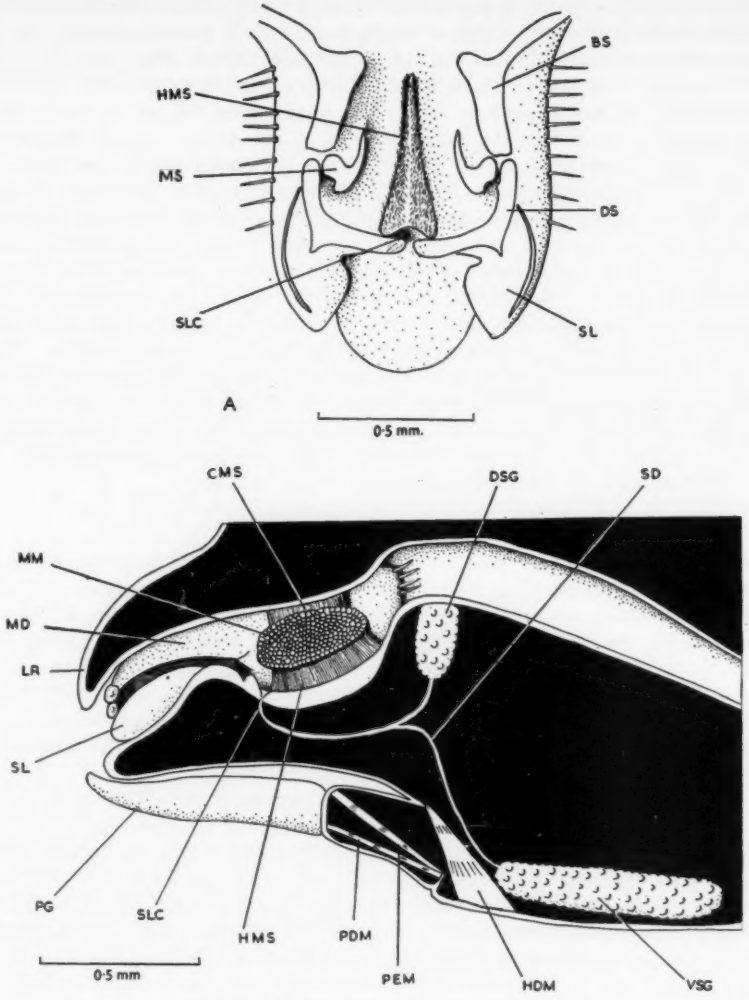


Fig. 5.—Hypopharynx.

A. Dorsal view of hypopharynx and superlingulae.

B. Lateral view of a median section through the head to show the relation between the mandible, hypopharynx and the cibarial roof.

BS, basal sclerite; CMS, cibarial molar setae; DS, distal sclerite; DSG, dorsal salivary gland; HDM, hypopharyngeal depressor muscle; HMS, Hypopharyngeal molar setae; LR, labrum; MD, mandible; MS, median sclerite; MM, molar area of mandible; PDM, paraglossal depressor muscle; PEM, paraglossal elevator muscle; PG, paraglossae; SD, Salivary duct; SLC, salivary cup; SL, Superlingulae; VSG. Ventral salivary gland.

The dorsal wall of the hypopharynx is largely composed of flexible cuticle. Towards the tip, the dorsal wall of the hypopharynx is strengthened by three pairs of V shaped sclerites composed of hard elastic cuticle (Figs. 5A, BS, MS, DS). Dorsally each superlingula is stiffened by a thin chitinous rod. Distally the underside of the hypopharynx has two longitudinal ridges, which fit into corresponding grooves in the upper surface of the dorso-ventrally flattened wedge-shaped labial paraglossae. Depression of the paraglossae is accompanied by that of the hypopharynx. The hypopharynx is mainly depressed by the paired hypopharyngeal depressor muscles (Figs. 5B and 9, HDM) which originate ventrally on the mentum and are inserted posteriorly on the underside of the hypopharynx. In the absence of any suitable muscles, elevation of the hypopharynx is due to haemocoelic fluid pressure. When the hypopharynx is elevated the basal sclerites (Fig. 5, BS) keep the hypopharyngeal sides flat and so elevation mainly occurs medially in the region of the molar setae. The distal sclerites (DS) articulate upon the median sclerites (MS) and are rotated forwards and upwards and so help to move food particles backwards to the posterior region of the cibarium. The median sclerites (MS) are small, form a base for the articulation of the basal and distal sclerites and give stability to the central region of the hypopharyngeal dorsal surface.

Labium. (Fig. 1C)

The underside of the head is covered by the labium. The submentum is large and the mentum is a transverse sclerite laterally bearing the two three jointed palps, one on each side and anteriorly the paraglossae, which are fringed with strong setae on their distal and median surfaces. The glossae are normally regarded as being absent in Dermaptera. The paraglossal depressor and elevator muscles (Fig. 5B, PDM, PEM) originate on the front wall of the mentum and are inserted respectively on the ventral and dorsal sides of the paraglossae. The palp is three jointed and the arrangement of the muscles is shown in Fig. 9.

The mentum is a narrow sclerite which can be slightly retracted under the front of the submentum by the mentum retractor muscles (Fig. 9, MRM) which originate posteriorly on the underside of the submentum and are inserted on the posterior wall of the mentum.

The submentum is an exceptionally large sclerite which forms the main median ventral wall of the head capsule. Posteriorly the submentum is joined to the neck by a rectangular gular sclerite of doubtful homology.

MOUTHPART MOVEMENTS. (Fig. 6)

During feeding the mandibles are rhythmically opened and closed and these movements are in phase with the elevation and depression of the labrum.

When the mandibles are closed (Fig. 6A), the maxillae are protracted and abducted, as are also the laciniae. As the mandibles open (Fig. 6B) the maxillae are raised and pulled inwards and slightly backwards. At the same time the laciniae galeae and palpi are bent inwards, and this is later accompanied by maxillary retraction. When the mandibles are wide open (Fig. 6C) the maxillae are completely retracted and abducted, but are protracted, rotated outwards

and distally depressed during mandibular closure (Fig. 6D). The movements of the maxillae are therefore out of phase with the movements of the mandibles During mandibular closure the labium and hypopharynx are elevated and the cibarial roof depressed : the opposite movements occurring as the mandibles reopen.

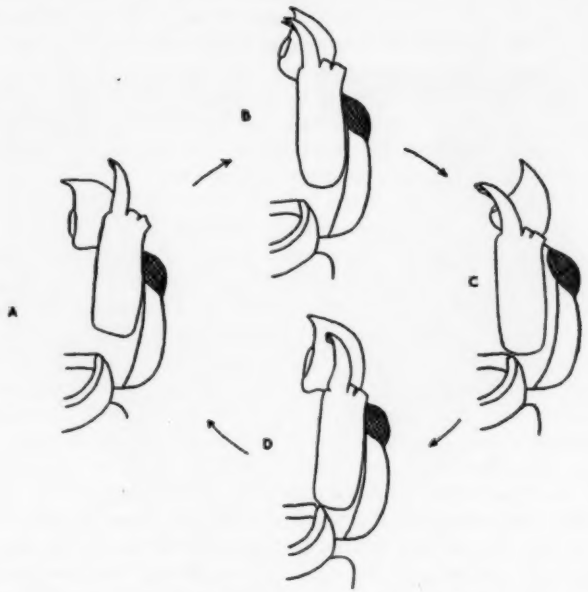


Fig. 6.—To show the movement of the left mandible maxilla and lacinia during feeding.

FEEDING

Forficula auricularia feeds upon plant and animal matter in the following manner. The head is raised from a hypognathous to a prognathous position, while the mandibles cut a series of transverse incisions in the food material. During closure the inwardly directed mandibular teeth bore a small tunnel for the lacinial teeth, while nearer the mouth the cutting edges shear off a layer of food substance. If the two mandibular incisions are close together the food particle is cut free and falls on to the dorsal side of the hypopharynx. Some food particles are loosened and freed through being bruised or crushed. If the two consecutive mandibular incisions are more widely spaced, the slices of food matter will remain attached distally to the bulk of the food material and another process is, therefore, needed to cut them free and transport them to the mouth. This function is performed by the maxillae, which have a horizontal rotatory action described above.

During mandibular opening the head is thrust forwards and the opened lacinial teeth are driven into the substratum between two previous mandibular

incisions. The maxillae are then pulled towards each other and by a chisel-like action of the lacinial teeth a semi-circular disc of food matter is encircled and cut free ready to be drawn upwards and backwards into the cibarial cavity by the retracting maxillae: the hypopharynx at this stage being depressed ready to receive the newly freed food particle. Meanwhile the labrum is lowered and the labium raised and the food is pushed on to the upper surface of the shovel-like hypopharynx. The orally directed setae on the labral edge and distal surface of the hypopharynx encourage the movement of the food material towards the mouth.

The passage of food through the cibarium cannot be observed directly, but the sequence of events described below is based upon deductions from the mouth-part movements and the structure of the organs concerned, on the assumption these function with maximum efficiency.

Distally the inner surfaces of the laciniae are vertical and face backwards, becoming increasingly medially and dorsally directed towards the base. During maxillary closure, therefore, newly freed food particles are forced by the laciniae on to the upper surface of the depressed hypopharynx. Here they are salivated as they pass over the salivary cup to be masticated between the closing mandibular molar surfaces. It would appear that at this time the cibarial roof is depressed by contraction of the bands of transverse muscles (Fig. 4B, CTM) and the hypopharynx is raised. If this is so then the molar setae on the cibarial roof and hypopharynx move respectively downwards and upwards and aid mastication. Saliva from the salivary cup is drawn upwards between the hypopharyngeal setae by capillarity. As the mandibles open, masticated food particles are sucked towards the oesophagus by contraction of the cibarial and oesophageal dilator muscles (see Figs. 4B and 7). As the mandibles close a second time the orally directed setae on the median posterior corners (see Figs. 4A, 4B and 5B) push the remaining food particles into the oesophagus. The head is then raised slightly while the mandibles open and the retraction of the maxillae is completed. During mandibular closure the maxillae are rotated outwards and the laciniae and galeae opened.

It will be seen, therefore, that the mouthparts of *Forficula* are so constructed that they can only function effectively when the head is *raised*, and this is in contrast to many Orthoptera and lepidopterous larvae which can only feed at the leaf edge when the head is *lowered*. In these latter insects leaf sections are cut free by the mandibles and then forced upwards through the cibarium into the oesophagus by head depression. In these insects the maxillae play little part in food collecting, beyond holding the leaf edge between the mandibles.

THE CRANIAL NERVOUS SYSTEM (Figs. 4B, 7 and 10)

The cranial nervous system of *Forficula auricularia* is in many respects similar to that of *Locusta migratoria* as described by Albrecht (1953), but there are also some interesting differences. The brain of *Forficula* is rotated postero-dorsally so that the protocerebra lie posterior and slightly dorsal to the main body of the brain mass. The protocerebra give rise laterally to the optic nerves, which run posterior to the dorsal arms of the tentorium, to the compound

eyes. A pair of antennal nerves (Fig. 7, AN) arise from the deutocerebra, and run antero-laterally to the antennal sockets, giving off small fibres to the antennal muscles (Fig. 7, AM) before entering the antennae. From the tritocerebrum, paired nerves run forwards to join the median frontal ganglion (Fig.

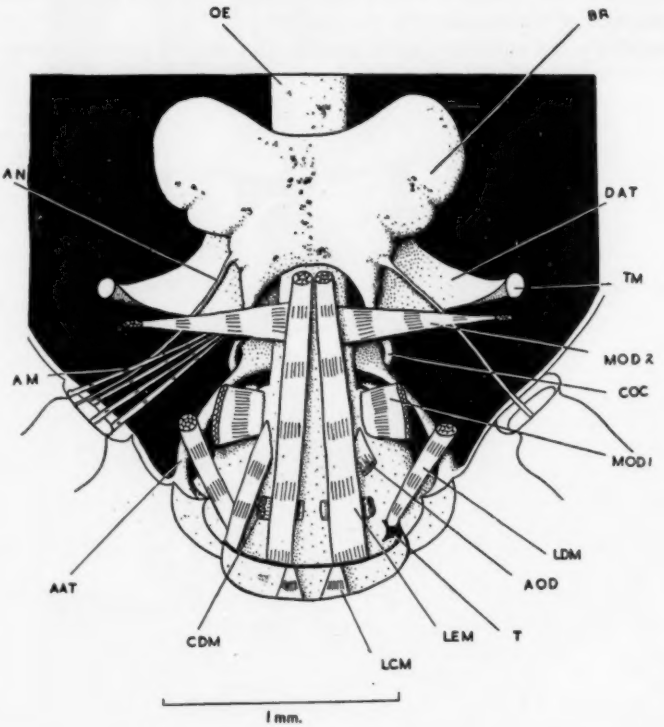


Fig. 7.—Dorsal view of the anterior region of the head capsule to show the cibarial muscles. The cuticle forming the roof of the head capsule is treated as though it were transparent.

AAT, anterior arm of tentorium; AM, antennal muscles; AN, antennal nerve; AOD, anterior oesophageal dilator muscle; BR, brain; CDM, cibarial dilator muscle; COC, circumoesophageal commissure; DAT, dorsal arm of tentorium; LCM, labral compressor muscle; LDM, labral depressor muscle; LEM, labral elevator muscle; MOD1, first median oesophageal dilator muscle; MOD2, second median oesophageal dilator muscle; OE, oesophagus; T, tormae; TM, tentorial macula.

4B, FG) from which nerves run to cibarial muscles and the tip of the labrum (LRN). The brain is joined by a circumoesophageal commissure to a large ventral suboesophageal ganglion, which is curved upwards anteriorly. The suboesophageal ganglion anteriorly gives rise to a pair of mandibular nerves,

which run forwards to the mandibles, and give rise to a lateral branch which runs backwards under the tentorium to innervate the mandibular muscles situated in the rear of the head capsule. (Fig. 4B, MNN). The maxillary nerves (Fig. 4B, MXN) also arise from the front of the suboesophageal ganglion, but are slightly ventral to the mandibular nerves. The labial nerves arise ventrally near the posterior end of the ganglion and then run forwards near the underside of the ganglion to the labium.

Posteriorly the suboesophageal ganglia give rise to paired nerves, which lead backwards to the thoracic ganglia.

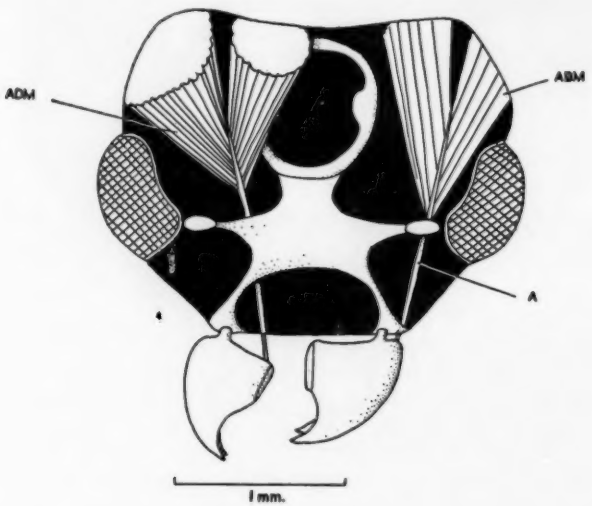


Fig. 8.—Dorsal view of the head to show the mandibular muscles. (The roof of the head capsule is treated as though it were transparent.)
A, apodeme; ABM, abductor muscle; ADM, adductor muscle.

In addition to these main nerves there is a small post-oesophageal commissure, which arises laterally from the two trito-cerebra and forms a loop under the oesophagus. Ventro-laterally this loop gives rise to fine nerve fibres which innervate the ventral oesophageal dilator muscles. Fine paired nerves run from the hypocerebral ganglia which anteriorly gives rise to a recurrent nerve which runs forwards medially under the brain to the frontal ganglion.

Compared with those of *Locusta* and *Periplaneta* the brain and suboesophageal ganglia are relatively enlarged and seem to be associated with the complex social habits and the specialised feeding movements described above.

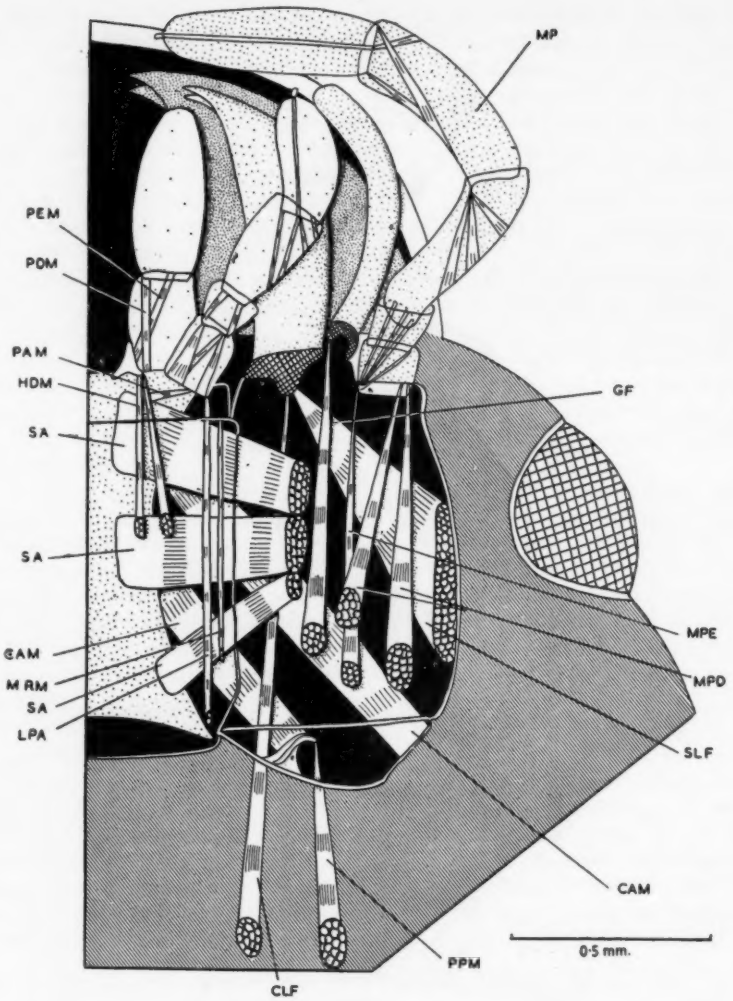


Fig. 9.—Musculature of the maxilla and labium as seen from the ventral side of the head capsule. The surface cuticle is treated as though it were transparent.

CAM, cardo adductor muscles; CLF, cranial lacinial flexor muscle; GF, galeal flexor muscle; HDM, hypopharyngeal depressor muscle; LPA, abductor muscle of labial palp; MP, maxillary palp; MPD, depressor muscles of maxillary palp; MPE, elevator muscle of maxillary palp; MRM, mentum retractor muscle; PAM, adductor muscle of labial palp; PDM, paraglossal depressor muscle; PEM, paraglossal elevator muscle; PPM, posterior promotor muscle; SA, stipes adductor muscles; SLF, stipes lacinial flexor muscle.

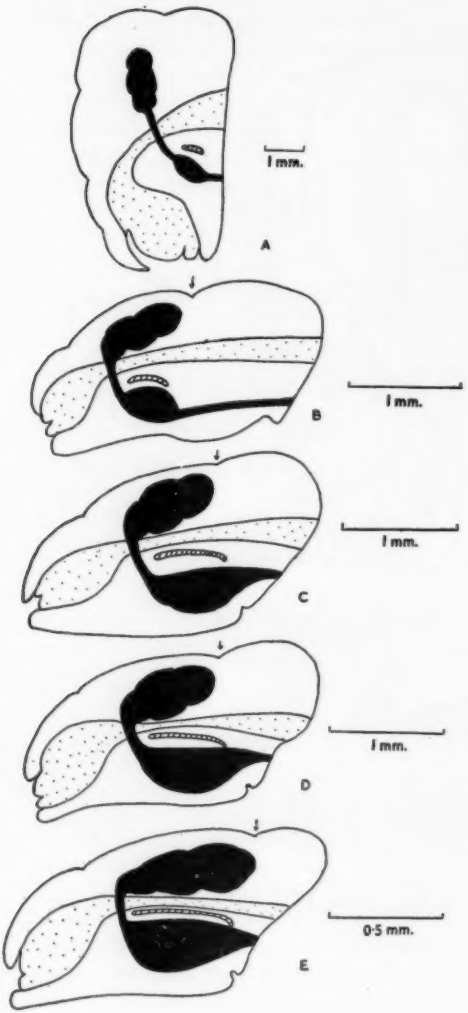


Fig. 10.—Lateral views of longitudinal sections, taken to the left of the middle line, to show the position of the brain, suboesophageal ganglion, and tentorial body in *Locusta migratoria* (Orthoptera) and four earwigs. Nervous system shown in black. Cibarium and oesophagus dotted, and tentorial body diagonal lines.

A, *Locusta migratoria*; B, *Cranopygia cumingi* (Pygidicranidae); C, *Epilabis penicillata* (Labiduridae); D, *Forficula auricularia* (Forficulidae); E, *Labia minor* (Labiidae).

THE NECK (Figs. 11 and 12)

The views of previous authors on the nature of the insect neck fall into five groups, which may be summarised as follows. Straus Durckheim (1828) and Verhoeff (1902) both thought the ventral cervical sclerites of *Forficula auricularia* were sterna of one or more vestigial segments between the prothorax and the head capsule. This region they called the microthorax, but subsequent embryological investigations have failed to find any evidence of the existence of these micro-thoracic segments and for that reason these views have little support amongst modern authors. Huxley (1877) and Comstock & Kochi (1902) held the view that the neck was derived from the labial segment : a view which is partly supported by the work of Smrecznski (1932) on the development of *Silpha*. Newport (1839) suggested that the neck was a detached portion of prothorax and although later papers of Crampton (e.g. 1926) seem to favour this interpretation, this author (1909 and 1917) had previously held that the neck was an intersegmental region. The hypothesis, which best fits the known facts is due to Snodgrass (1935) who has proposed a combination of the last three views, by suggesting the neck is derived from both the labial and prothoracic segments and would, therefore, also contain an intersegmental region.

It is perhaps premature to discuss the exact homology of the insect neck, because it is functionally one of the most important parts of the insect body, in as much as it contains the muscles, which move the head capsule relative to the thorax. Head movements vary considerably from one order to another and are associated with feeding habit differences. Thus the carnivorous mantids and Odonata are able to move the head through a wide range of angles, whereas many grasshoppers can only move the head up and down—the anterior flange of the front of the prothorax reducing side-to-side head movement to a minimum. In *Locusta* the ventral cervical sclerites are absent and the dorsal and ventral musculature is well developed, whereas in *Periplaneta*, which has an opisthognathous head, the ventral musculature is relatively reduced. In *Forficula* the neck has a two-fold function of supporting a prognathous head and also allowing it to move in various directions. The differences between the necks of Dermaptera and Orthoptera are further emphasised by the fact that the musculature of the two orders is not easily mutually homologised ; the cervical nerves are derived from two ganglia in the Orthoptera but only the prothoracic ganglia in the Dermaptera. Whether the necks are, therefore, strictly homologous in these two orders is a matter for further investigation.

FUNCTIONAL MORPHOLOGY OF THE NECK (Figs. 11, 12 and 13)

The neck of *Forficula auricularia* is a short horizontal cylinder with dorsally inclined oblique ends. Anteriorly at the base of the labium the neck is stiffened by a broad rectangular sclerite known as the gular (Fig. 11, GS). The ventral cervical surface is also strengthened by two large ventral cervical sclerites : the posterior being the larger (Fig. 11, PVS). The arthrodial membrane between the gular and the anterior cervical sclerite (Fig. 11, AVS), the anterior and posterior ventral sclerites and between the posterior ventral sclerite and the prosternum is folded, in each case, under the front of the more posterior sclerite.

Masson stained sections of the neck show the sclerites to be composed of a thin epicuticle and equal thicknesses of exo-and endo-cuticle. Groups of sensory setae are present and probably supply the insect with sense data concerning the movements and position of the head. On the ventral side of the neck, where the arthrodial membrane is folded transversely under the

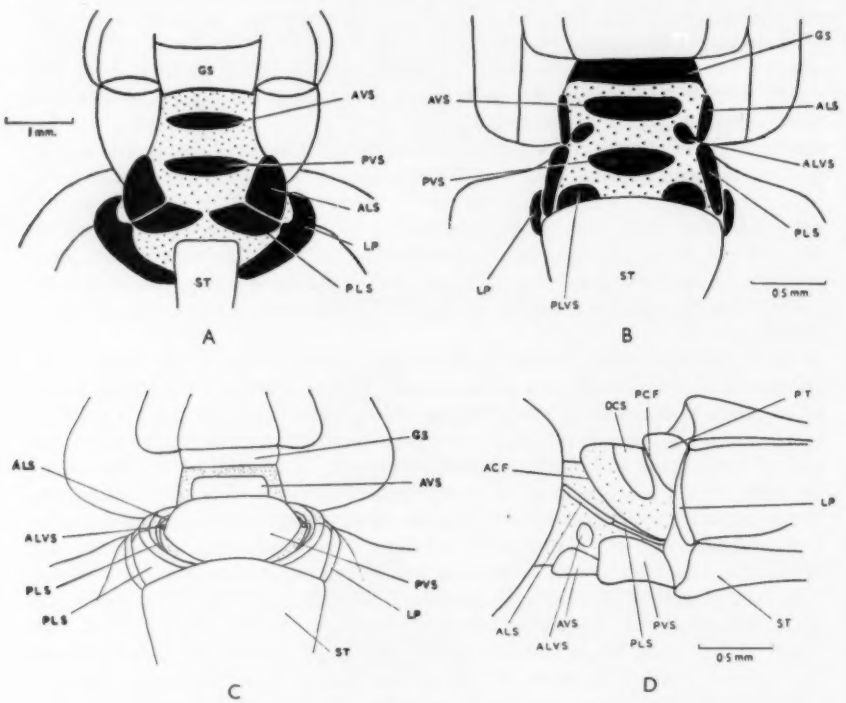


Fig. 11.

A. Ventral view of the neck of *Periplaneta americana* L.
B. Ventral view of the neck of *Cranopygia marmoricrura*.
C. Ventral view of the neck of *Forficula auricularia*.
D. Diagrammatic view of the lateral surface of the neck of *Forficula auricularia*.

ACF, anterior cervical fold; ALS, anterior lateral sclerite; ALVS, anterior latero-ventral sclerite; AVS, anterior ventral sclerite; DCS, dorso lateral sclerite; GS, gular sclerite; LP, lateral plate; PCF, posterior cervical fold; PLS, posterior lateral sclerite; PLVS, posterior latero-ventral sclerite; PT, pre-tergum; PVS, posterior ventral sclerite; ST, prothoracic sternum.

ventral sclerites, the arthrodial membrane is raised into transverse folds and ridges (Fig. 12B), the distal slope being steeper than the proximal one. This arrangement of sclerites and arthrodial membrane gives support and rigidity to the underside of the neck and also permits a large measure of expansion and contraction. Dorsally, immediately posterior to the head capsule are two

transverse folds (shown Fig. 11D and in the neck of *Epilabis penicillata*, Fig. 25, ACF, PCF). These folds run transversely dorsally and then run obliquely backwards on the sides of the neck to the lateral margins of the prothoracic sternum. The anterior fold is immediately dorsal to the lateral cervical sclerite and anterior to the dorsal cervical sclerite. The posterior fold (PCF) is mainly confined to the dorsal side of the neck, but is extended laterally as a shallow groove anterior to the lateral plates (Figs. 11 and 25, LP). Posterior to the second fold and immediately anterior to the prothoracic tergum is a short transverse sclerite—the pretergum. The lateral cervical arthrodial membranes are composed of relatively thin exocuticle and a much thicker endocuticle which is strongly laminated. The surface of the arthrodial membrane is raised into numerous small flattened domes and here the exocuticle is slightly thicker (Fig. 12A). The domes are present where the cuticle may be folded or rolled in several directions, but are absent on the underside of the neck, where the cuticle is folded transversely. If the sides of a fold are in mutual contact, the domes of one side tend to lie between those of the other and so form a simple interlocking device.

On each side of the neck there is a small auxillary sclerite (the dorso-lateral sclerite Fig. 25, DLS) between the anterior cervical fold and the dorsal cervical sclerite. A sclerite situated between the posterior cervical fold and the pleural sclerite of the prothorax is best regarded as homologous with the lateral plate of *Periplaneta* (Cf. Figs. 11A, B and C, LP).

The thin anterior lateral cervical sclerite (Fig. 11C, ALS) articulates anteriorly with the occipital condyles, but posteriorly is fused to the posterior lateral cervical sclerites to form a more or less rigid bar, which articulates posteriorly with the antero-lateral corners of the prothorax. The lateral cervical sclerites are hidden by the ventral extensions of the upper side of the anterior cervical fold, which is posteriorly stiffened by two posterior lateral sclerites. A comparison of the necks of *Periplaneta* (Fig. 11A) and *Cranopygia marmoricrura* (Serville) (Pygidicranidae) (Fig. 11B) shows this sclerite to be a lateral extension of the posterior lateral cervical sclerite. Henson (1950) regards this sclerite as the second or posterior lateral cervical sclerite and therefore interprets the two lateral cervical sclerites as being only the anterior sclerite. Henson's views are based upon a comparison of the necks of *Periplaneta* and *Forficula*, whereas a clearer picture of the homologies of the lateral cervical sclerites is given by a comparison of the necks of other Dermaptera, especially the primitive Pygidicranidae. If the nomenclature suggested above is adopted, it has the added advantage that the posterior ventral longitudinal muscle would have the same points of origin and insertion in both *Forficula* and *Periplaneta*.

Between the posterior ventral cervical sclerite and the posterior lateral sclerite there is a small auxillary sclerite—the anterior latero-ventral sclerite (Fig. 11, ALVS) (the juxta sclerite of Crampton). In *Cranopygia cumingi* (Dohrn) there is also a small posterior latero-ventral sclerite just in front of the prester-

num, but in *Forficula* this sclerite is either absent or has become incorporated into the posterior lateral sclerites, as a median extension.

In *Locusta* and *Periplaneta* the head is moved mainly up and down and is supported by the two pairs of lateral cervical sclerites to which are attached powerful muscles for moving the head. In *Forficula* the head is more mobile

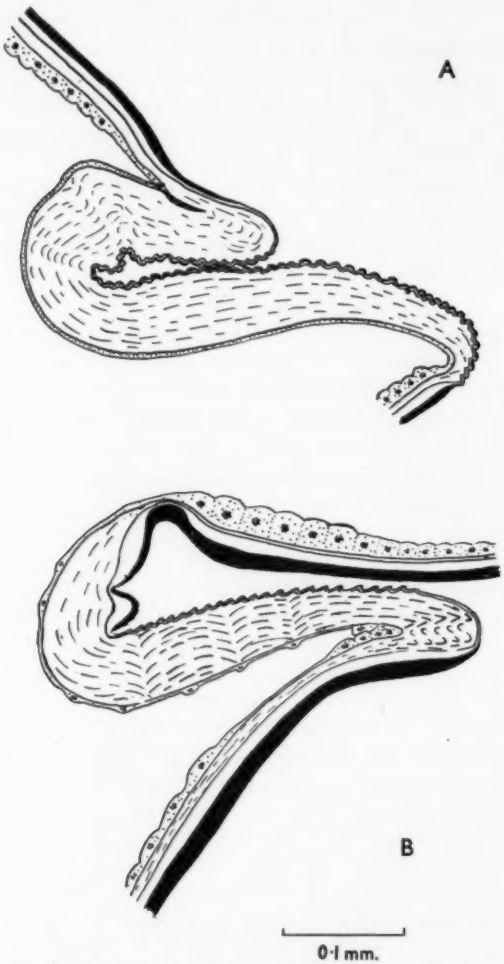


Fig. 12.—Sections of cervical cuticle to show the structure of the arthrodial membrane.
A, is taken from the lateral region of the neck and shows the domes on one side of the fold lying between the domes on the other to form an interlocking mechanism.
B, is taken from the ventral side of the neck between the first and second ventral cervical sclerites and shows the arthrodial membrane with a series of superficial transverse ridges.

but as the lateral cervical sclerites are thin and fused together and their rôle in head support is, therefore, unlikely to be large. The neck is, however, capable of being extended by haemocoelic fluid pressure and when slightly contracted the sides of the cervical folds become interlocked and form a temporary head support while feeding occurs. By neck extension the lateral folds are reopened and a new temporary support can be made to hold the head in another position.

Once the head is clamped in a given position it can be moved up or down by the following muscles : The head is turned forwards and upwards from a hypognathous to a prognathous position by the contraction of (Fig. 13) :—

(1) *The dorsal longitudinal muscles.*
These originate on the anterior face of the first dorsal phragma and are inserted on an apodeme at the base of the anterior dorsal fold.

(2) *The protergal muscles of the head.*
These are bands of muscles on either side of the neck and thorax which are inserted on an apodeme at the base of the first dorsal fold and originate medially and laterally on the pronotum.

The following two muscles raise the lateral cervical sclerites and the side of the neck, and therefore indirectly raise the head.

(3) *Cephalic muscles.*
A pair of large muscles, which originate, one on each side on the dorsal membrane of the neck immediately behind the head and are inserted on the anterior region of the posterior cervical sclerite and the inner surface of the anterior fold.

(4) *The pre-tergal muscles.*
A pair of muscles, which originate on the pre-tergum and are inserted on the posterior end of the posterior lateral cervical sclerites.

As the head is raised forwards and upwards, the arthrodial membrane under the anterior margins of the ventral cervical sclerites and prosternum is unrolled, while the length of the dorsal side of the neck is reduced by rolling arthrodial membrane into the two dorsal folds. When the head is lowered into the hypognathous position the opposite occurs. The head is lowered into a more hypognathous position by the contraction of :

(5) *The ventral longitudinal muscles.*
A pair of muscles, divided on each side, into a dorsal and ventral bundle of fibres, which originate on the apophysis of the prosternum and are inserted laterally on the posterior tentorial bridge.

(6) *The anterior ventral longitudinal muscles.*
A pair of thin muscles, which originate on the posterior tentorial bridge, pass between the dorsal and ventral bundles of the ventral longitudinal muscles and are inserted medially on the posterior margin of the posterior ventral cervical sclerite.

(7) *The posterior ventral longitudinal muscles.*
A pair of muscles, which are inserted on the posterior end of the posterior lateral cervical sclerite and originate on the prothoracic apophysis.

The head can be turned sideways by the contraction of muscles Nos. 2, 6 and 7 on one side of the neck. Other muscles helping to turn the head are :

(8) *The protergal muscles of the neck.*
A pair of muscles which originate on the sides of the episternum and are inserted on the neck membrane lateral to the occipital condyles.

(9) *The pleural neck muscles.*
A pair of muscles which originate on the pleural suture of the prothorax and are inserted on the cervical membrane on the same side just ventral to the protergal muscles.

During turning the arthrodial membrane is rolled into the dorsal folds on one side of the neck and unrolled from the folds on the other.

Rotation of the head is achieved by contraction of the appropriate muscles on the opposite side of the neck, the main muscles concerned being Nos. 8 and 9. The position and arrangement of the dorsal folds in the cervical membrane allows cranial rotation to take place.

CERVICAL NERVE SUPPLY (Fig. 13)

In *Forficula* and *Cranopygia* the suboesophageal ganglion sends fibres to the prothoracic ganglion by a pair of thin nerves which lie lateral to paired ventral nerve cords. As these lateral nerves (Fig. 13, LN) approach the prothoracic ganglion, they come to lie adjacent to a bundle of nerve fibres coming out of the ganglion. Branches of this lateral cervical nerve innervate muscles Nos. 5, 6 and 7 and also send nerve fibres to the sensory setae on the ventral side of the neck. A lateral cervical nerve arises from the prothoracic ganglion and runs forwards and outwards median to the protergal and pleural neck muscles (Nos. 8 and 9) and innervates the muscles on the dorsal side of the neck.

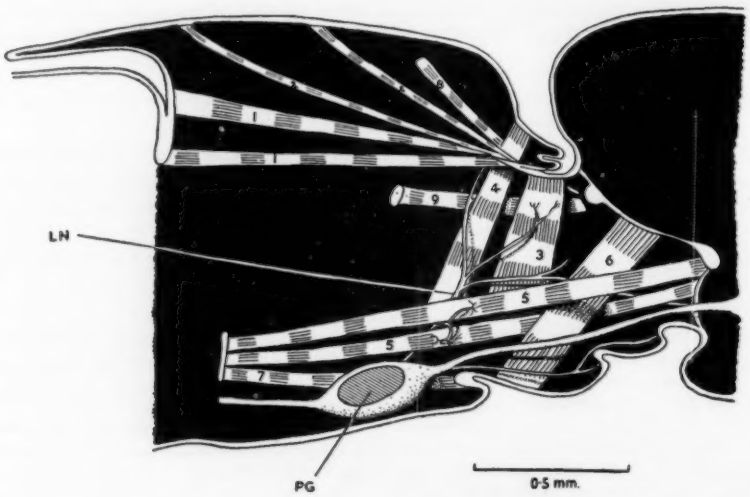


Fig. 13.—View of the left side of the neck to show arrangement of muscles (see text) and the nerve supply from the prothoracic ganglion.
LN, lateral cervical nerve; PG, prothoracic ganglion.

In *Locusta* and *Periplaneta*, however, there is an anterior cervical nerve, which arises from the suboesophageal ganglion and innervates the dorsal side of the neck. These differences may indicate that the ontological origins of the necks of Dermaptera and Orthoptera are not identical, but it must also be remembered that in Insecta, muscles from a given segment are innervated by

nerve fibres from the ganglion of the same segment as well as from fibres which arise from axons in the previous anterior ganglion.

ACTION OF THE HAEMOCOELIC FLUID

It has been shown above that neck extension, maxillary abduction and depression, and promotion of the laciniae, galeae and maxillary palps as well as the elevation of the hypopharynx and paraglossae are all accomplished, at least in part, by haemocoelic fluid pressure. Cannon (1947) has shown that the extension of the thoracic appendages of the barnacle *Lithotrya* occurs by the circulation of blood through a series of blood spaces within the limbs, but as the cavities of the mouthparts of *Forficula* are open to the general haemocoel, this possibility is excluded. It would appear, therefore, that the maxillae are normally abducted and protracted, the maxillary palps, galeae and laciniae abducted, and the hypopharynx and labium extended, by haemocoelic fluid pressure which acts in antagonism to the contraction of the muscles of these appendages.

Blood is pumped forwards by the dorsally placed heart into a blood sinus anterior to the brain. Fluid is prevented from flowing backwards dorsally and laterally by connective tissue between the brain and the roof of the cranium, the dorsal arms and the pleurostomal sutures. Blood therefore, can, only flow sideways round the gut immediately posterior to the internal opening of the mouthparts. Blood flows backwards ventral to the sub-oesophageal ganglion and the ventral diaphragm. From this ventral blood sinus fluid flows into the perivisceral cavity and from thence into the pericardium.

It can be demonstrated that haemocoelic fluid is involved in the mouthpart movements as follows. Pressure on the abdomen produces neck extension, maxillary promotion, depression and abduction, and also hypopharyngeal and labial extension. If the anterior end of the submentum is depressed so as to drive fluid backwards, the maxillae remain closed and retracted, while the hypopharynx and paraglossae become depressed, though this latter movement may be due to the mechanical pressure applied. Pressure on the rear of the submentum drives fluid forwards and is followed by maxillary promotion and abduction as well as the opening of the laciniae, galeae and maxillary palpi. If the arthrodial membrane between one of the maxillae and the labium is cut longitudinally with a fine blade, pressure on the rear of the submentum causes maxillary movement on the uncut side only and escape of fluid on the other. If a cut is made in the side of the thorax so as to allow haemocoelic fluid to escape, the abdomen telescopes. The abdomen contracts in a similar manner when an earwig is kept under dry conditions, but becomes extended when the insect is given an adequate supply of water. It would appear, therefore, that the abdomen is largely responsible for maintaining a haemocoelic fluid pressure and an account of the structure of the abdomen therefore, is, relevant to the study of earwig feeding habits.

It is significant to note that the mouthpart movements listed above occur during mandibular closure and that at this time there are sometimes also slight constrictive movements of the abdomen. It would appear that mouthpart movements are indirectly controlled by the abdomen. In the feeding methods

described above the hypopharynx, galeae and laciniae are moved very quickly during abduction to take up new positions preparatory to cutting free another section of food material. The time available for these abductive movements is short and it is at this moment that the elasticity of the sclerotised bases of the laciniae and galeae play an important part in accelerating the movements of these mouthpart appendages.

STRUCTURE OF THE ABDOMEN (Figs. 14 and 15)

The abdomen is composed of ten segments, the pygidium usually being interpreted as the vestiges of the 11th segment. The first abdominal tergum is incorporated into the thorax and the great development of the hind coxae is associated with the absence of the first abdominal sternum. The abdominal segments are inclined forwards causing the terga and sterna to alternate in lateral view. The terga extend on to the underside of the abdomen and laterally contain the spiracles showing that they are partly composed of sclerotised pleural areas. In the male there is a separate tergum and sternum for the 2nd to the 9th segments inclusive, those of the 10th being fused into a

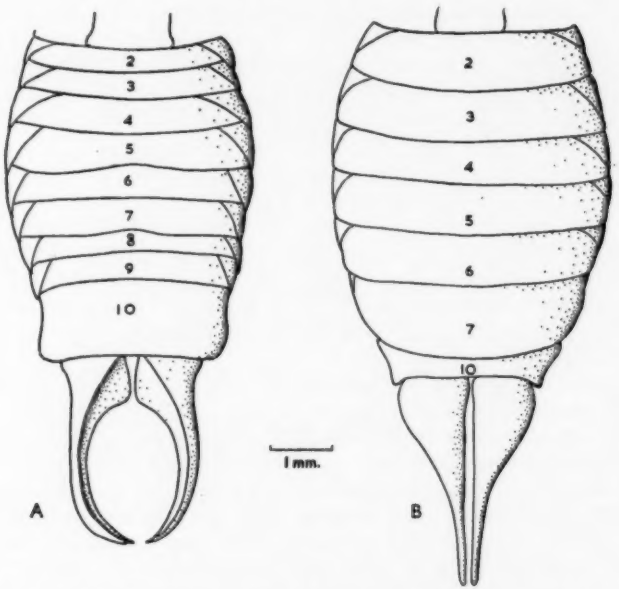


Fig. 14.—Ventral views of the abdomens of male (A) and female (B) specimens of *Forficula auricularia*. Sternites numbered.

hard cuticular ring on which are borne the cerci. In the female the 8th and 9th tergites and sternites are reduced to narrow transverse sclerites and are normally hidden under the terga and sterna of the previous segments. The terga and sterna are greatly elongated and overlap the next two to four segments. In

Forficula the tergites and sternites anteriorly bear a vertical apodeme for the origins of the abdominal muscles. The sclerites of the abdomen fit over one another like the sections of a telescope and the abdomen has, therefore, a wide range for extension and contraction while the staggering of the sterna and terga allows the abdomen to be bent or twisted as necessity requires. Omitting the musculature of the first abdominal segment and of

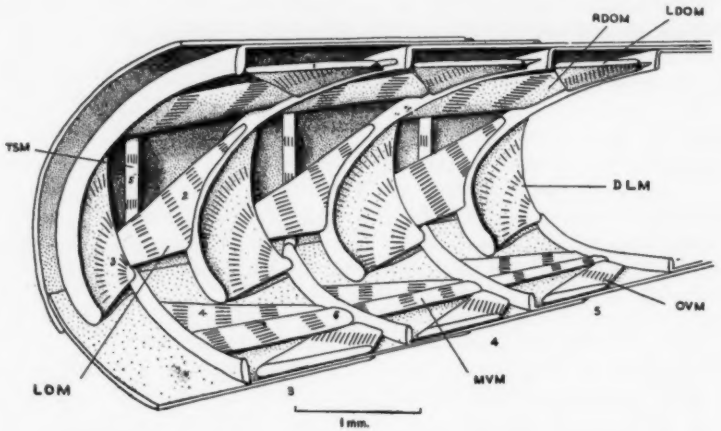


Fig. 15.—Diagrammatic view of the musculature on the right side of abdominal segments 3, 4 and 5.

DLM, dorso-lateral muscle; LDOM, left dorsal oblique muscle; LOM, lateral oblique muscle; MVM, median ventral muscle; OVM, oblique ventral muscle; RDOM, right dorsal oblique muscle; TSM, tergo-sternal muscle.

the genitalia for consideration elsewhere, the movements of the abdomen are due to the contraction of the muscles of segments 2 to 9 inclusive and their action may be summarised as follows. (Cf. Fig. 15).

The abdomen is bent upwards by the contraction of the following muscles :

(1) *The paired dorsal oblique muscles* originate laterally on the posterior surface of one of the tergal apodemes and are inserted medially on the anterior surface of the tergal apodeme of the next posterior segment.

(2) *The paired lateral oblique muscles* originate laterally and ventrally on the posterior surface of the tergal apodeme of one segment and are inserted on the anterior surface of the tergal apodeme of the next posterior segment.

(3) *The paired dorso-lateral muscles* originate laterally on the posterior surface of the tergal apodeme of one segment and are inserted ventrally on the anterior surface of the next posterior tergal apodeme.

The abdomen can be bent sideways by the contraction of these muscles on one side and are assisted by the contraction of one of each of the following pairs of muscles.

(4) *The paired oblique ventral muscles* originate laterally on the posterior surface of the sternal apodeme of one segment and are medially inserted on the anterior face of the sternal apodeme of the next posterior segment.

(5) *The paired tergo-sternal muscles*, which originate laterally on the sternal apodeme of one segment and are inserted laterally on the tergum of the same segment.

The abdomen is bent downwards by the contraction of muscles 4 and 5 on both sides as well as of the

(6) *Paired median ventral muscles*. These muscles originate on the posterior face of the sternal apodeme of one segment and are inserted on the anterior face of the sternal apodeme of the next posterior segment.

The abdomen is shortened by the combined action of muscles 1, 2, 3, 4 and 6 listed above. When this occurs the segments become telescoped and the sclerites of one segment slide easily inside those anterior to it. When the abdomen is extended the segments are eliptical in cross section, but become circular as the abdomen contracts in length. At the same time the terga and sterna are forced apart and muscles No. 5 are extended. The sclerites are composed of elastic cuticle and tend to curl to form a longitudinal cylinder when separated from the body. Contraction of muscles No. 5 therefore pulls against the "spring" of the tergites and sternites, pulls the tergites and sternites towards each other and the abdomen therefore extends under haemocoelic fluid pressure. For this reason muscles 1 to 4 and 6 are antagonistic to muscles No. 5.

THE HEADS AND NECKS OF OTHER DERMAPTERA

The range of dermapteran heads and necks can best be explained with reference to a number of types, which have been selected upon the advice of Dr W. D. Hincks of the Manchester Museum, a well known authority on the order. The types are selected to be as typical as possible of the families and subfamilies to which they belong and are illustrated in Figs. 17 to 28.

DISCUSSION

The hypognathous type of insect head is usually regarded as primitive in as much as the mouth parts preserve the ventro-lateral position of ambulatory appendages (Snodgrass, 1935). While admitting these facts, Walker (1932) has suggested that the heads of the early Pterygota may have been prognathous. In support of his thesis Walker drew attention to the widespread occurrence of prognathism amongst primitive and fossil insect orders as well as in *Grylloblatta*, which in view of its affinities with several insect orders, is considered to have a primitive level of pterygote organisation. Indeed Walker went so far as to claim that *Grylloblatta* fits almost exactly the hypothetical ancestor to the Dermaptera and the Saltatoria. Carrying his thesis to its logical conclusion Walker was obliged to suggest that the hypognathous condition in the Orthoptera and other orders was not primitive, but secondarily acquired. Walker's ideas of the Dermaptera were mainly based upon *Forficula auricularia*, which is one of the most specialised of earwigs. Had he been able to examine some of the primitive Pygidicranidae he might have written differently.

If Walker's hypothesis is correct, it follows that the reflexed brains of the Forficulidae (Fig. 10D) and Labiidae (Fig. 10E) are to be regarded as more primitive than the simpler level of cranial organisation to be found in the Pygidicranidae (Fig. 10B) or the Orthoptera such as *Locusta* (cf. Fig. 10).

Even if this difficult position can be accepted, Walker's ideas are not easily applied to the Dermaptera as a whole. The form of the head capsule and neck of *Grylloblatta* show greater affinities to the slightly prognathous heads of the Pygidicranidae than the more prognathous species of the Forficulidae and Labiidae. A more reasonable explanation would be that prognathism is not a primitive pterygote feature and that *Grylloblatta*, the Pygidicranidae and the Orthoptera have evolved from a common hypognathous ancestor perhaps not unlike the generalised type described by Snodgrass (1928 and 1935).

Other considerations support this view. The mouthparts of *Forficula* and other earwigs can only function effectively when the head is gradually raised from a hypognathous to a prognathous position, whereas, the mouthparts of *Locusta* and other Orthoptera can only function effectively when the head is lowered. In spite of these functional differences there is a great similarity between the arrangements of the muscles in the locust head, as described by Albrecht (1953), and the head of *Forficula* described above—a fact which implies some affinity between the two insects. Both types of head could be derived from an intermediate form, which fed by moving its hypognathous head slightly in either direction : a condition to be found in modern blattids and mantids.

In insects the hypognathous type of head is primitively associated with herbivory. True, the carnivorous Mantidae and Odonata have a hypognathous head, but in these insects the thoracic limbs (especially those of the prothorax in mantids) are specially adapted for holding the prey during feeding. It seems, therefore, that the early Pterygota were probably herbivorous : an assumption which is not unreasonable in view of their initial appearance in the Carboniferous period.

It follows, therefore, that those Dermaptera which show the greatest affinities with other primitive hypognathous Exopterygota are likely to show a level of organisation from which other Dermaptera evolved and it is in this connection that the Pygidicranidae are of special interest. This family was established by Verhoeff (1902) and in 1915 Burr included in it those Dermaptera with an abdomen ending in three plates, with double penis lobes in the males and gonapophyses in the females of most species. These are certainly primitive Pterygote features and it is significant that these earwigs show a greater affinity with the Orthoptera and Dictyoptera than any other family of Dermaptera. For example the head is slightly prognathous, the mouth parts of the pygidicranine type are very generalised and the neck is very blattoid, in consisting of arthrodial membrane ventrally supported by two small cervical sclerites. The absence of these sclerites in the Orthoptera is doubtless associated with the need for the ventral side of the neck to be flexible and the specialised feeding methods of these insects.

There is a general similarity in the arrangements of the cranial and cervical muscles of all the Dermaptera examined. This implies that the head and mouthparts are moved in a similar way. There is also a similarity in the arrangements of the muscles of the mouthparts of Dermaptera and *Locusta*, the latter having a slight maxillary rotatory action, though not as pronounced as the former. The trends towards prognathism, shown by the Pygidicranidae are best

interpreted as adaptations for carnivory. The transition from herbivory to carnivory has been a gradual one and the majority of earwigs show some degree of plant feeding. Taking the Orthoptera as a possible starting point it seems reasonable to suggest that the early earwigs captured their prey between their mandibular teeth and pulled the food into the cibarium by a rotatory action of the maxillae. Prey capture would be facilitated if the head were simultaneously raised slightly so as to enable the maxillae to obtain a better grasp of the prey. Here then, in embryo, would exist the combination of mouthpart movements which when developed could become the complex movements described above in *Forficula*. At an early stage there must have been special modifications in the head capsule to increase the efficiency of this feeding method.

The herbivorous type of insect mandible found in the Orthoptera may be regarded as primitive so far as the Pterygota are concerned. It is characterised by being thick and broad, the distal teeth short and the proximal molar area broad. With the adoption of carnivory the distal teeth became longer and tended to point forwards. The mandible also became flattened and thinner distally to enable the insect to feed in crevices in the vegetation or under bark and in soil, while the molar area naturally remained relatively thick. But movements of such a large flat blade were probably not easily controlled and the surrounding medium might tend to press them to one side and so prevent the mandibles being used with any degree of precision. Mandibular closure upon a prey would tend to force the points of articulation outwards. The former difficulty seems to have been solved by folding the cranial edge round the base of the mandibles to form a most efficient grip and the latter by an anterior enlargement and infolding of the genae at the sides of the cranium to form the system of internal struts associated with the mandibular condyles and shown in Fig. 1D. Increased maxillary movement was accompanied by an elongation of the body of the tentorium to accommodate the origins of the maxillary muscle, while a general elongation of the underside of the head capsule between the bases of the mandibles and maxillae increased the distance for maxillary protraction and retraction, and at the same time made the head capsule slightly prognathous.

A carnivorous feeding habit led to an enlargement of the mandibular adductor muscles for crushing and killing the prey and was accompanied by a lateral and posterior expansion of the genae on which these muscles originate. So great was the expansion of the genae that the ventral head margin became withdrawn beneath the cranium and the maxillae, therefore, were moved backwards and forwards in two longitudinal ventral cranial grooves between the inflated genae and the labium. The expansion of the genae and occiput also resulted in the eyes and antennal sockets being relatively nearer the mouthparts, though there was doubtless a forward shift in the position of the sense organs as a compensation for prognathism.

These evolutionary changes from the primitive pterygote head lead to a form of head capsule not unlike that found in the pygidicranine type (Fig. 17), though this is certainly specialised in having very large eyes and posteriorly inflated genae. A better idea of the primitive dermapteran head and neck

can be obtained from a synthesis of the primitive characters shown by various pygidicranid types. On this basis it seems reasonable to suggest that in many respects the generalized dermapteran is not unlike the anataeliine type in having a long head, eyes flush with the general head surface, the antennal sockets dorsally placed (see Fig. 16 G, H and J) and the frontal sutures anteriorly

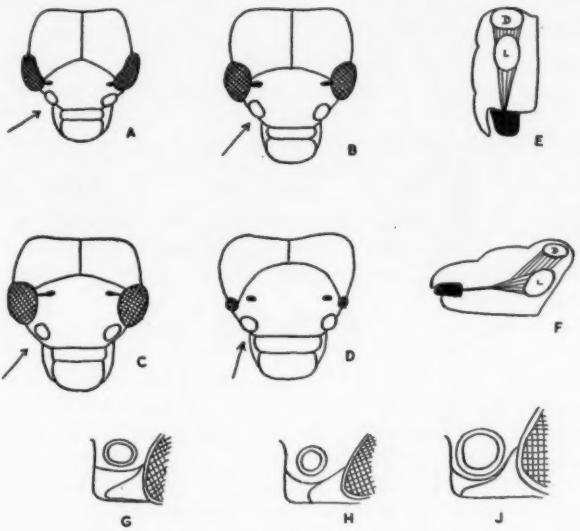


Fig. 16.

A. Dorsal view of the head of *Cranopygia marmoricrura* (Pygidicranidae).
B. Dorsal view of the head of *Labidura riparia* (Pallas) (Labiduridae).
C. Dorsal view of the head of *Forficula auricularia* (Forficulidae).
D. Dorsal view of the head of *Chaetospania pittarellii* (Borelli) (Labiidae).
Arrows on the left side indicate direction of antennal muscles.
The head shows the eyes, antennal sockets, frontal and epicranial sutures and tentorial maculae.
E. Lateral view of the adductor mandibular muscles in the head of *Locusta*.
F. Lateral view of the adductor mandibular muscles in the head of *Forficula*.
D—dorsal fibres.
L—lateral fibres.
G. Lateral view of the head of *Anataelia*.
H. Lateral view of the head of *Esphalmenus*.
J. Lateral view of the head of *Bormansia*.
These three figures show the relative positions of the antennal sockets and the subocular and pleurostomal sutures.

placed. The mouthparts would be short and generalized as in the pygidicranine type (Fig. 17 B, C and D), while the neck would be blattoid-like, as in both types. The cylindrical body of the diplatyine type would appear to be a more primitive condition than the dorso-ventrally flattened bodies of the majority of Dermaptera.

General trends in the evolution of the dermapteran head (Figs. 10 and 16)

Before discussing the relationships between the main types of earwigs, it will be helpful to survey the main trends in the evolution of the head. This is illustrated with reference to an example from each of the four main families as follows. The Pygidicranidae is represented by *Cranopygia cumingi*, the Labiduridae by *Labidura riparia* and *Epilabis penicilliata*, the Forficulidae by

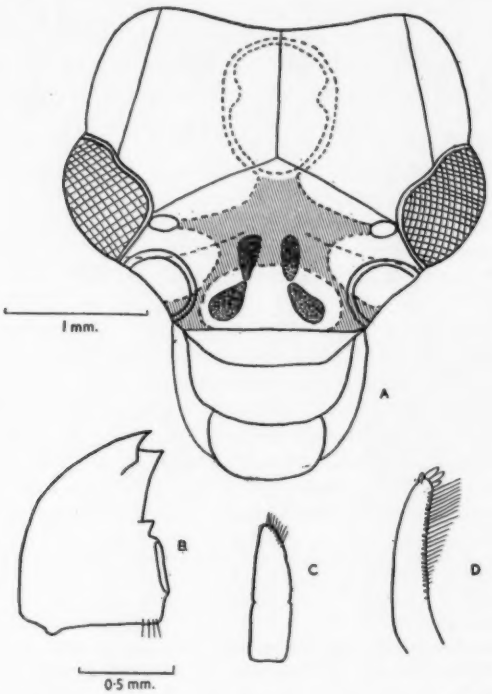


Fig. 17.—Pygidicranine type. Example—*Cranopygia marmoricrura* (Serville).
A. Dorsal view of the head with the positions of the foramen magnum and tentorium shown by dotted lines and by mechanical stipple. B. Left mandible. C. Left lacinia. D. Left paraglossa.

Forficula auricularia and the Labiidae by *Labia minor*, and *Chaetospania pittarellii*.

Starting with the primitive Pygidicranidae, there is a general trend for the dermapteran head to become increasingly prognathous. This reaches a maximum in the Labiidae and is originally associated with an increasingly carnivorous diet. In the hypognathous head of *Locusta*, the mandibles are pulled towards the mid-line by the paired adductor muscles. Each muscle is attached to the mandibular base by an apodeme and is composed of dorsal and lateral bundles (Fig. 16E, D and L). The dorsal fibres originate on the

epicranium and the lateral fibres on the genae. Between the bundles of muscle fibres passes the dorsal tracheal trunk. As the dermapteran head becomes prognathous the cranium is bent upwards anterior to the frontal sutures. This means that the lateral muscle fibres remain in the same plane as the movement of the mandibles and the efficiency of the muscles is in no way reduced as the head becomes prognathous. On the other hand the dorsal muscle fibres become increasingly inclined to the plane of the mandibular movement and as the angle increases the horizontal component of the contractile force steadily decreases (Fig. 16F). Under these conditions the anterior and more vertical muscle fibres tend to disappear and the epicranial sclerites, upon which the muscle fibres originate, decrease in length, and the posterior oesophageal dilator muscle (Fig. 4B, POD), which originates at the anterior median corners of the epicranial sclerites is posteriorly displaced. Fig. 16 A, B, C and D show the effects of this change, namely the epicranial sclerites are shortening and the frontal sutures gradually taking a more posterior position. The posterior shift in the oesophageal dilator muscles also makes room for the brain to become more and more posteriorly and dorsally rotated and this is well illustrated by Fig. 10 B, C, D and E : the position of the frontal sutures being indicated by a small arrow. Snodgrass (1947) has discussed at some length the significance of the position of the frontal and epicranial sutures in a number of insects and concludes " the shift in the lines from one position to another affects the manner of ecdysis, but the cranial structure is, thereby no more altered than is an orange changed in structure according to the way it is peeled ". True the position of the sutures has a functional significance at ecdysis, but the sclerites must also act as the origins of muscles from one ecdysis to the next as well as in the adult which does not moult at all. In the life cycle of most insects the time devoted to moulting is relatively small.

Fig. 10 also shows that as one passes from the pygidicranine to the labiid type there is a gradual increase in the size of the brain and particularly in the size of the suboesophageal ganglion : changes which are possibly associated with the movement of the mouth parts during feeding. There is also a gradual elongation of the head anterior to the suboesophageal ganglion (cf. Fig. 10B–E). This is due to a gradual increase in the length of the distal segments of the mouthparts as well as an enlargement of the hypopharynx. These changes are functionally associated with the rotatory movements of the maxillae for food collecting and Fig. 10 also shows that there is a corresponding increase in the length of the tentorial body upon which the maxillary muscles originate.

In the Orthoptera the dorsal tentorial arms are situated ventral to the optic nerves and dorsal to the antennal nerves. As one passes from the pygidicranine to the labiid type there is a gradual dorso-posterior rotation of the brain and the dorsal arms of the tentorium are therefore displaced posteriorly. This is shown in Fig. 16 by a gradual posterior shift in the position of the tentorial maculae. In the pygidicranine type they are situated near the front of the eyes, being at mid eye level in the labidurid type, near the rear of the eyes in the forficulid type and actually posterior to the eyes in the labiid type *Chaetospania*. As the antennal muscles originate on the base of the tentorial arms they run transversely across the head capsule in *Locusta*

but tend to take a more longitudinal path as one passes from the primitive to the more specialized earwigs. The change in the direction of the antennal muscles is indicated in Fig. 16 A, B, C and D by the change in the direction of the arrows.

In the Orthoptera, the antennae are median to the eyes and in the primitive anataeliine type the antennal sockets are likewise situated on the front (now dorsal) side of the head (Fig. 16G). As the head becomes increasingly dorso-ventrally flattened the antennal sockets shift on to the side of the head and the angles between the subocular and anterior pleurostomal sutures decreases, while the angle between the dorsal and ventral arms of the pleurostomal sutures is reduced. These changes are illustrated by Fig. 10G, H and J taken from the anataeliine (Fig. 18), the esphalmenine (Fig. 22) and the karschielline (Fig. 23) types. The function of these changes is to increase the rigidity of the internal struts to the mandibular condyles as head flattening occurs.

Dermapteran types

The range of head and neck structure can best be explained with reference to a series of types, no evolutionary significance necessarily being implied. Since many of the Dermaptera appear to feed upon both animal and plant matter an odd observation upon the feeding habits of a given earwig may be misleading and unrepresentative of the species as a whole. Such fragmentary evidence as exists, however, agrees fairly well with the structure of the mouth-parts, which show a range of structural organization from one family to another, in spite of Burr's (1910) remark that there are no differences of any systematic value.

Closely approximating to the ancestral earwig head and neck is that of the pygidicranine type (Fig. 17). Its mouth parts are very generalised and the mandibles have acutely pointed and widely separated teeth. The maxillae are short and the laciniae end in blunt forwardly directed spines. The paraglossae are short and distally bear a row of stiff setae. The mouthparts differ from that of *Forficula* in not having the mandibular teeth directed medially, nor the laciniae and paraglossae adapted for tunnelling. The generalised form of the mouth parts in the pygidicranine type suggests omnivory, but unfortunately nothing is known of the feeding habits of these earwigs. The head is fairly primitive and the anterior position of the frontal sutures and tentorial maculae suggest that the brain shows dorso-posterior rotation. Longitudinal sections through the head (Fig. 10B) show this to be the case. The neck is very blattoid-like, the ventral cervical sclerites being thin transverse bands of cuticle, while the lateral cervical sclerites are well developed. There is no great elaboration of the cervical folds to form a collar-like structure as in *Forficula*. (Fig. 11B).

The pyragrine type (Fig. 20) can be derived from the pygidicranine type, by shortening the mandibular teeth and enlarging the molar areas of the mandibles. The laciniae end in four claw-like antero-medially directed setae, which could be used to rake food into the cibarial cavity. The form of the mouthparts seems to imply that these South American earwigs are mainly herbivorous, but as the maxillae have a position posterior to that in

most Dermaptera—the cardoes actually being posterior to the base of the labium, it seems unlikely this arrangement is a primitive one. On this evidence it is suggested that the pyragrine type represents a secondary return towards herbivory.

The anataeliine type (Fig. 18) closely approximates to the generalised type of earwig, but the mandibular teeth are directed forwards and the molar areas are reduced. De la Escalera (1922) claims that *Anataelia* is carnivorous and readily feeds upon flies in captivity. The anataeliine type has a blattoid like neck and may be regarded as intermediate between the pygidicranine type and the diplatyine type (Fig. 19). The diplatyine type represents a trend

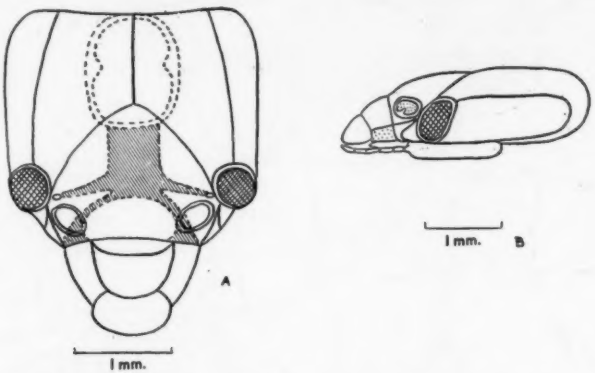


Fig. 18.—Anataeliine type. Example—*Anataelia canariensis* Bolivar.
A. Dorsal view of head. B. Lateral view of head.

towards predominate carnivory. The mandibles have large forwardly directed teeth ending in acute points (Fig. 19c) and the molar areas are relatively small. The maxillae are short and the lacinial teeth are spine-like and point forwards. The head has become more prognathous by an upward flexure of the anterior region of the head capsule at the level of the frontal sutures, making the frons distinct from the occiput. The genae and epicranial sclerites are elongated to accommodate the large mandibular muscles. These changes make the rear of the head capsule rectangular in transverse section. The eyes are very large and the tentorial maculae have become separated from the frontal and orbital sutures. The position of the frontal sutures would seem to indicate that little dorso-posterior cerebral rotation has taken place. Increased prognathism has led to an enlargement of the anterior ventral cervical sclerite to support the neck and cervical flexure now occurs mainly at the rear of the neck.

Burr (1910) believed the Diplatyinae to be predominately carnivorous, while Hincks (1955) upon the authority of Gravely states that *Diplatys gladiator* feeds upon mayflies on the walls in Calcutta. The crops of the two specimens available for study contained a preponderance of arthropod remains. The

general form of the mouthparts points to a predominately carnivorous feeding habit. The echinosomatine type (Fig. 21) is primitive in many respects. The forms of the mouthparts and neck are not specialised, yet the eyes are greatly enlarged and the posterior position of the frontal sutures indicates

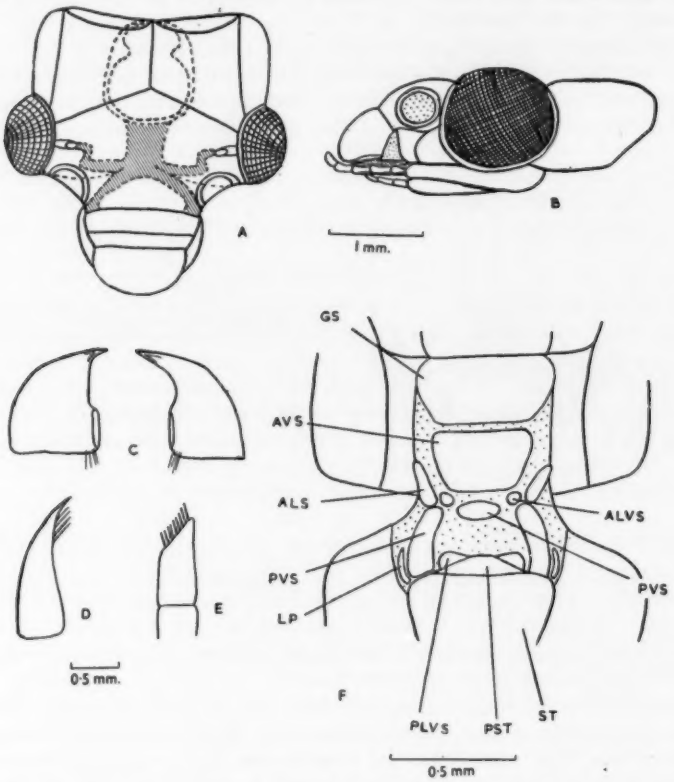


Fig. 19.—Diplatyine type. Example—*Diplatys macrocephalus* (Palisot de Beauvois).

A. Dorsal view of the head. B. Lateral view of the head. C. Mandibles. D. Left lacinia. E. Right paraglossa. F. Ventral side of the neck.

ALS, anterior lateral sclerite; ALVS, anterior latero-ventral sclerite; AVS, anterior ventral sclerite; GS, gular sclerite; LP, lateral plate; PLS, posterior lateral sclerite; PLVS, posterior latero-ventral sclerite; PST, presternum; PVS, posterior ventral sclerite; ST, sternum.

there is a greater degree of cerebral rotation than in the pygidicranine type, from which it can be derived as a separate line without much relation to other dermapteran types.

The blandicine and esphalmenine types (Fig. 22) form intermediate stages between the pygidicranine and the karschielline types (cf Figs 22B, 22D and 23B). The karschielline type (Fig. 23) has

antero-medially directed sharp mandibular and lacinial teeth and Dr Hincks informs me that the crops of Karschiellinae often contain large numbers of small ant heads. It seems likely that the karschielline type represents another independent trend towards a predominately carnivorous feeding habit. The posterior shift in the dorsal tentorial arms, due to increased prognathism has carried them slightly medially along the frontal sutures.

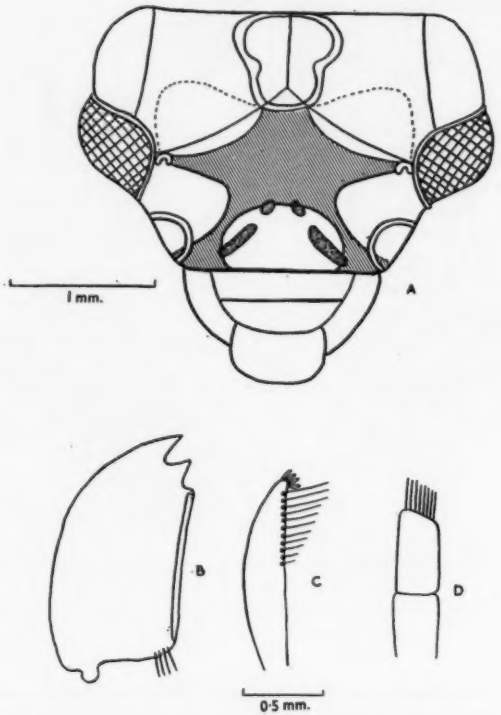


Fig. 20.—Pyragrine type. Example—*Pyragra fuscata* (Serville).
A. Dorsal view of the head. B. Left mandible. C. Left lacinia. D. Left paraglossa.

Increased prognathism has also led to an enlargement of both ventral cervical sclerites (Fig. 23F, AVS, PVS) to produce a type of neck which is found in no other group of earwigs.

The main trends towards complete carnivory lead from the pygidicranine type to the labidurid type (Fig. 24), the form of the mouthparts in general being similar to those of the karschielline type described above, though this may be due to parallel evolution. The genae of the labidurid type are elongated to accommodate powerful mandibular muscles. There is also a lengthening of the distal segments of the maxillae and labium, while increased maxillary

mobility is reflected in the lengthening of the tentorial body upon which the maxillary muscles originate (Fig. 10c). There is also increased cerebral rotation and the frontal sutures are here situated at mid eye level. In the neck the posterior ventral cervical sclerite (Fig. 25, PVS) has greatly enlarged for neck support, while the lateral cervical sclerites are reduced and their function is being taken over by a collar-like structure, similar to that described

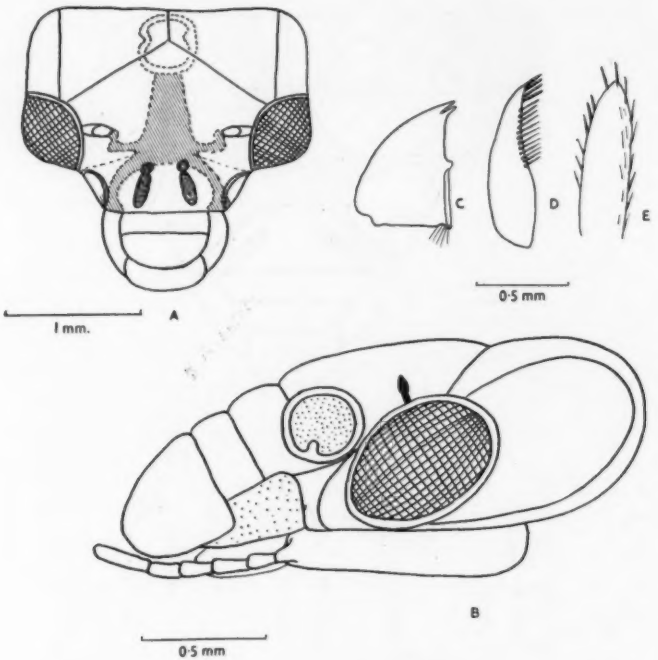


Fig. 21.—Echinosomatine type. Example—*Echinosoma sekelavum* Bormans. A. Dorsal view of the head. B. Lateral view of head. C. Left mandible. D. Left lacinia. E. Left paraglossa.

in *Forficula*. Such a neck can be made either rigid or flexible as required and seems to be an adaptation to feeding in narrow and restricted spaces, such as might occur in soil, litter or under bark.

The form of the mouthparts suggests carnivory and Burr (1910), Brindley (1918) and Lucas (1920) all state that *Labidura riparia* is mainly carnivorous, while Clausen (1940) states that it is partial to the larvae of the cotton worm. Both Williams (1931) and Zimmerman (1948) claim that *Euborellia annulipes* (Fig. 24 E–H) feeds predominantly on leaf hoppers in Hawaii. The series *Labidura-Euborellia-Allostethus*, while all being carnivorous labidurids, show a posterior shift in the position of the frontal sutures indicative of a gradual increase in cerebral rotation. The apachyine type (not figured) is little more than an

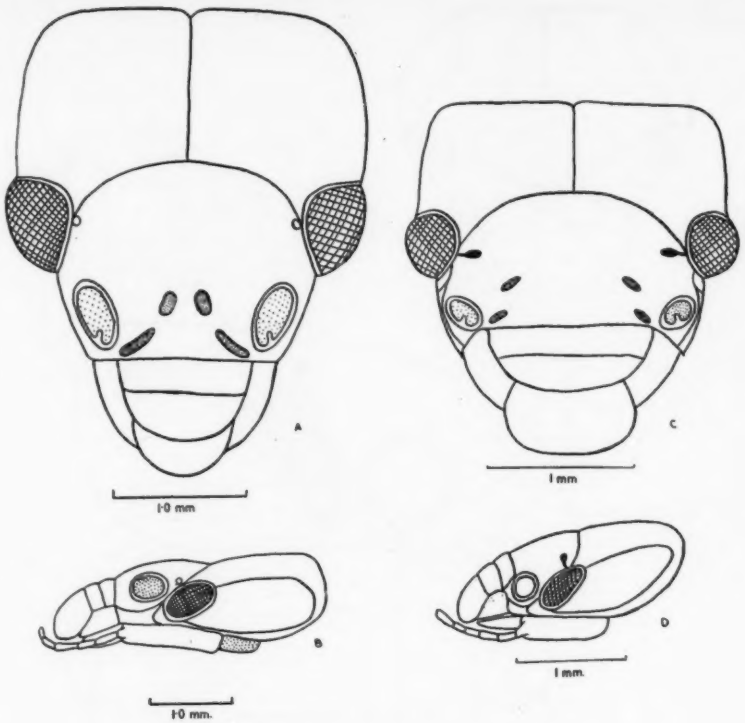


Fig. 22.—Blandicine type. Example—*Blandex solvendus* Burr.
A. Dorsal view of head; B. Lateral view of head.
Esphalmenine type. Example—*Esphalmenus lativentris* (Philippi).
C. Dorsal view of head; D. Lateral view of head.

extremely dorso-ventrally flattened labidurid type adapted to carnivory under bark. The labiid types can be derived from the Labidurid types as follows. The series *Nesogaster*, *Labia*, *Prosparatta* and *Chaetospania* (Fig. 26) show the mandibular teeth gradually becoming flattened and blade-like, while the distal segments of the maxillae and labium become greatly elongated and are brought as far forwards as possible (Fig. 10E). At the same time the tentorium elongates and the posterior shift in the position of the frontal sutures (Fig. 16D) is associated with complete rotation of the brain over the greatly enlarged suboesophageal ganglion. The dorsal arms of the tentorium are situated at the rear of the eyes in *Prosparatta* and to a greater extent in *Chaetospania*. There is a reduction of eye-size in these earwigs, the tentorial maculae losing contact with the frontal and ocular sutures. The antennal sockets are now directed forwards and the antennal muscles take a longitudinal rather than a transverse course, through the head capsule.

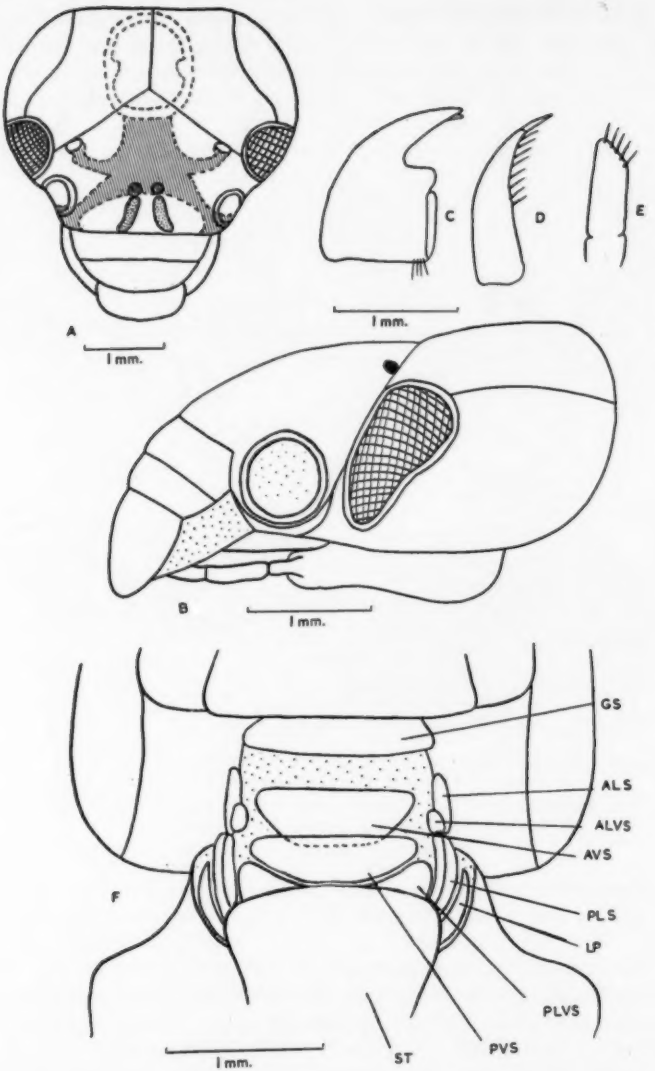


Fig. 23.—Karshielline type. Example—*Bormansia africana* Verhoeff.

A. Dorsal view of head; B. Lateral view of head; C. Left mandible; D. Left lacinia; E. Left paraglossa; F. Ventral view of neck.

ALS, anterior lateral sclerite; ALVS, anterior latero-ventral sclerite; AVS, anterior ventral sclerite; GS, gular sclerite; LP, lateral plate; PLS, posterior lateral sclerite; LPVS, posterior latero-ventral sclerite; PVS, posterior ventral sclerite; ST, sternum.

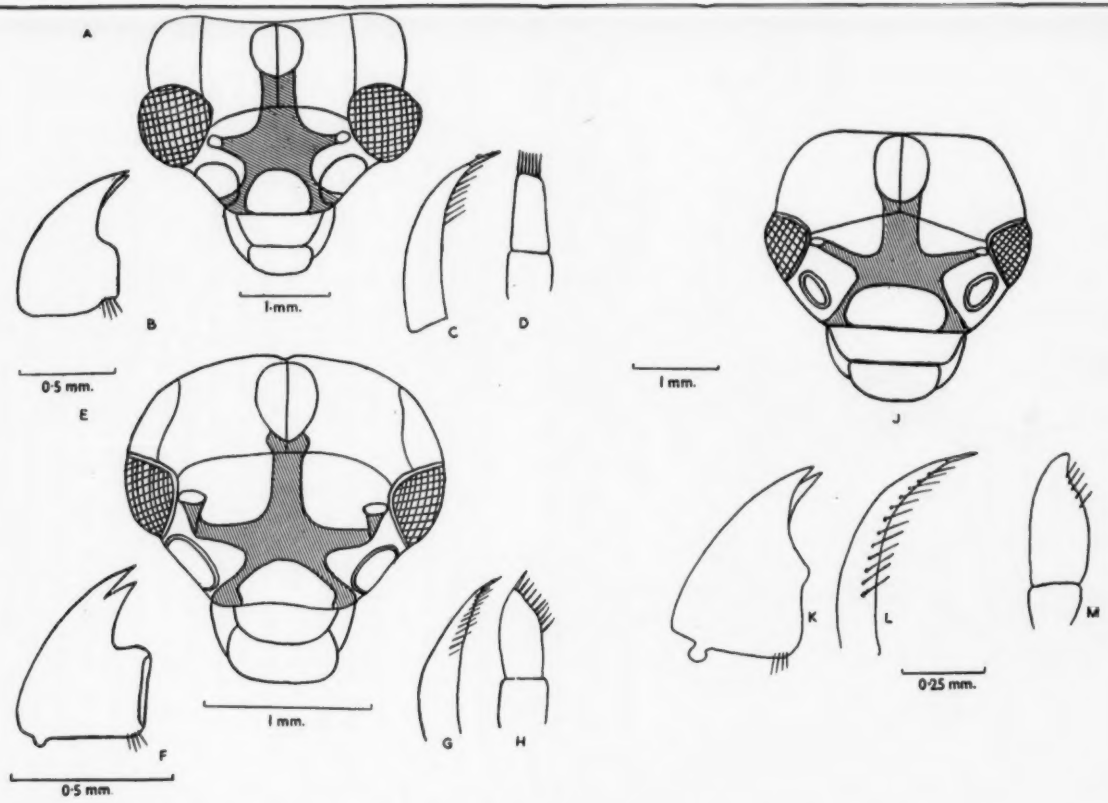


Fig. 24.—Labidurid types. Main example—*Labidura riparia* (Pallas). A. Dorsal view of head; B. Left mandible; C. Left lacinia; D. Left paraglossa. Subsidiary types. (a) *Euborellia annulipes* (Lucas). E. Dorsal view of head; F. Left mandible; G. Left lacinia; H. Left paraglossa. (b) *Allostethus indicum* (Burmeister). J. Dorsal view of head; K. Left mandible; L. Left lacinia; M. Left paraglossa.

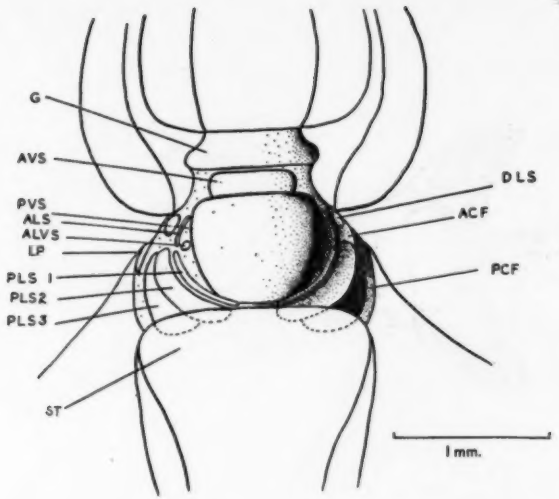


Fig. 25.—Labidurid type of neck. Example—*Epilabis penicillata* (Borelli).
ACF, anterior cervical fold; ALS, anterior lateral sclerite; ALVS, anterior latero-ventral sclerite; AVS, anterior ventral sclerite; DLS, dorso-lateral sclerite; GS, gular sclerite; LP, lateral plate; PCF, posterior cervical fold; PLS, 1, 2, 3, posterior lateral sclerites; PVS, posterior ventral sclerite.

The thorax of *Labia minor* shows a greater degree of dorso-ventral flattening. than in earwigs of other families. The thoracic segments slant forwards at a steeper angle and the metathoracic coxae are relatively elongated. These features suggest that the hind legs are the main organs of locomotion for pushing the insect through compact humus. *Labia minor* is common on dung heaps and occurs some distance below the surface where the material is fairly compact. The crops of the few specimens examined contained an abundance of arthropod remains, but one specimen also contained large numbers of pollen grains. Lucas (1920) records this insect as feeding readily upon flies. The combination of mandibular form, the elongation and forward position of the distal segments of the maxillae and labium, the reduction in the size of the eyes, the forwardly directed antennae, and the extreme dorso-ventral flattening of the body suggest that the labiid type of earwig is adapted for carnivory in compact vegetation and is a semi-subterranean insect.

The head, mouthparts and neck of the chelisochid type (Fig. 27) so closely resemble that of the labidurid type that the two types could be associated together. Terry (1905), Perkins (1913), Risbec (1935), Hincks (1947) and Zimmerman (1948) are unanimous in stating *Chelisoches morio* (F.) is mainly a carnivorous feeder. The Chelisochidae, however, differ from the labidurid and labiid types in having the second tarsal joint produced and this would seem to be an adaptation for climbing plants. *Chelisoches morio* is particularly partial to aphids and leaf-hoppers, which occur on the stems and leaves of sugar

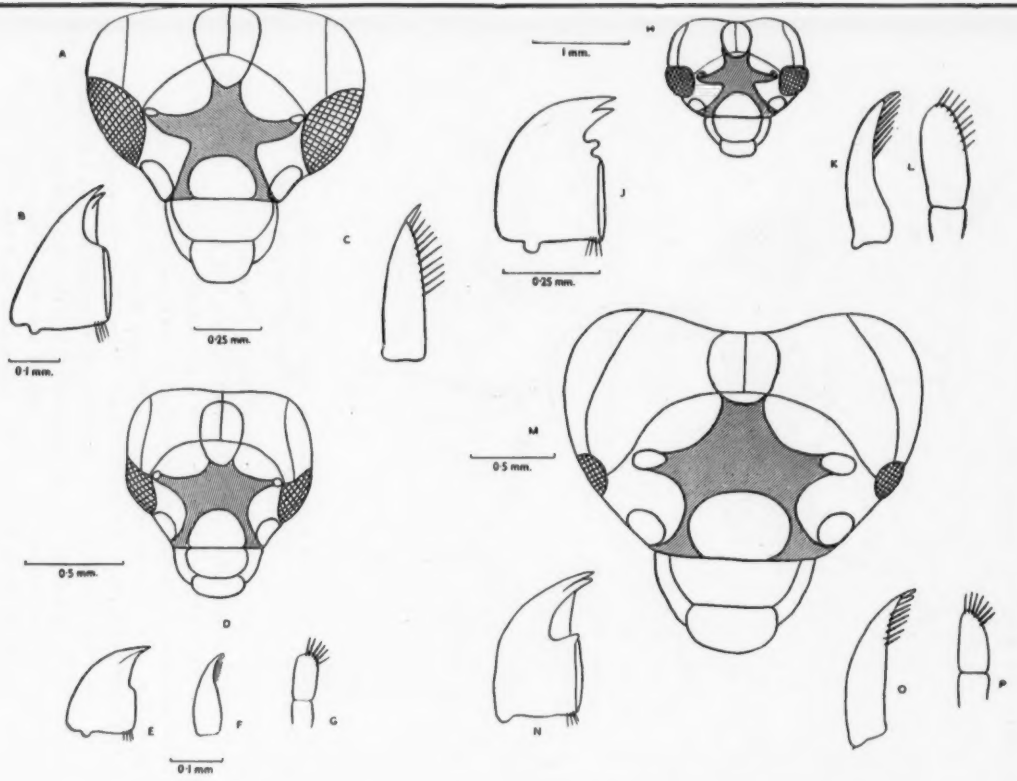


Fig. 26.—Labiid types. Examples—(A) *Nesogaster aculeatus* (Bormans). A, Dorsal view of head; B. Left mandible; C. Left lacinia; D. Left paraglossa. (B) *Labia minor* L. D. Dorsal view of head; E. Left mandible; F. Left lacinia; G. Left paraglossa. (C) *Prosparatta incerta* (Borelli). H. Dorsal view of head; J. Left mandible; K. Left lacinia; L. Left paraglossa. (D) *Chaetospania pittarellii* (Borelli). M. Dorsal view of head; N. Left mandible; O. Left lacinia; P. Left paraglossa.

cane. Risbec's observations that this insect feeds on leaf-mining larvae of hispid beetles are of particular interest, because they show how a carnivorous earwig could develop the tunnelling plant-feeding habits such as have been described above for *Forficula auricularia*.

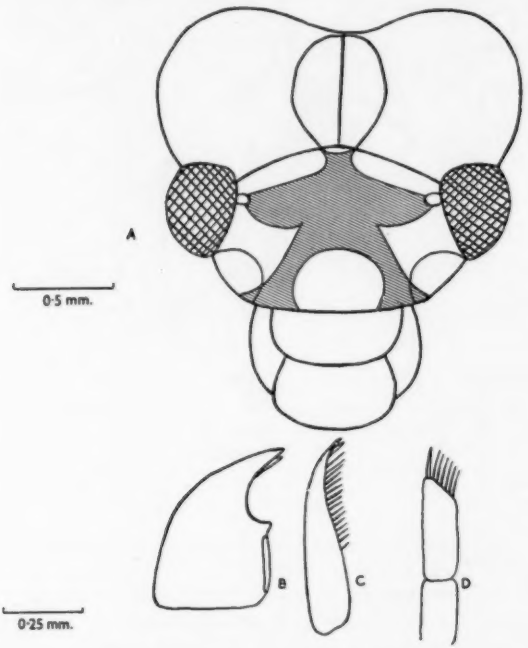


Fig. 27.—Chelisochid type. Example—*Chelisoches morio* (F).
A. Dorsal view of head; B. Left mandible; C. Left lacinia; D. Left paraglossa.

It has previously been stated that prognathism in Dermaptera is an adaptation to carnivory. In the main this statement is true but in the case of the forficulid types a correction is necessary. In this earwig type the acquisition of the prognathous condition has enabled the insect to tunnel into plant material, partially abandon carnivory and become mainly secondarily herbivorous.

In the forficulid types (Fig. 28A–M), the series *Doru–Forficula–Diaperasticus* shows the change in the form of the mouthparts as an adaptation to tunnelling feeding habits. This involves the elongation of the distal segments of the mouth parts and a lengthening and inturning of the mandibular teeth (Fig. 28B, E and K), the medial curvature of the laciniae so that the teeth take a more and more transverse position (Fig. 28C, F and L), while the paraglossal setae spread from the anterior to the inner margins (cf. Figs. 24D, 27D, 28D and 28M). As in the Labiidae increased prognathism is associated with the enlargement of the body of the tentorium and increased cerebral rotation, with the associated posterior shift in the positions of the frontal sutures. In

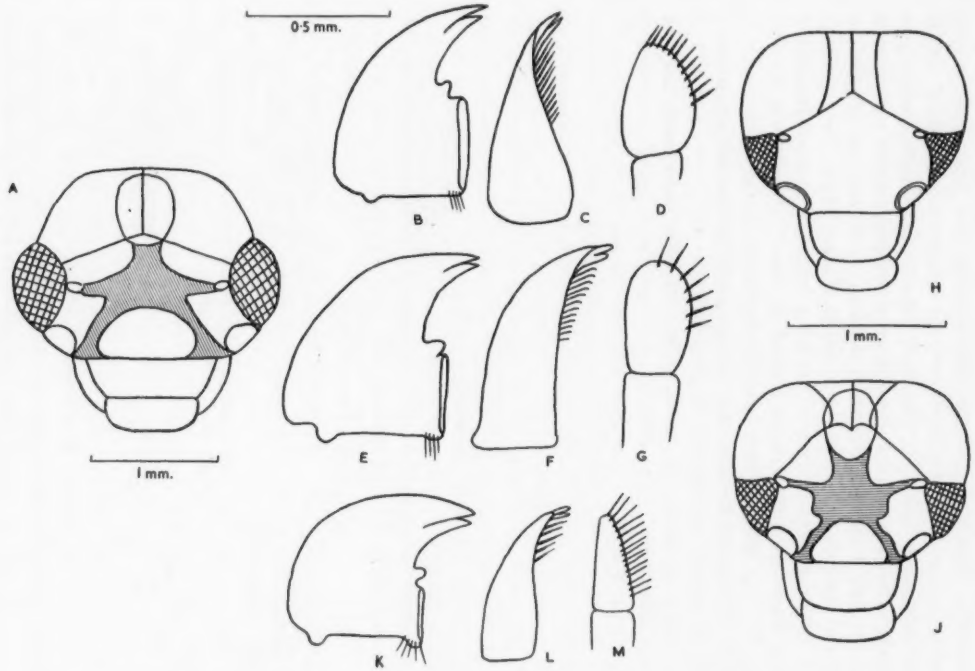
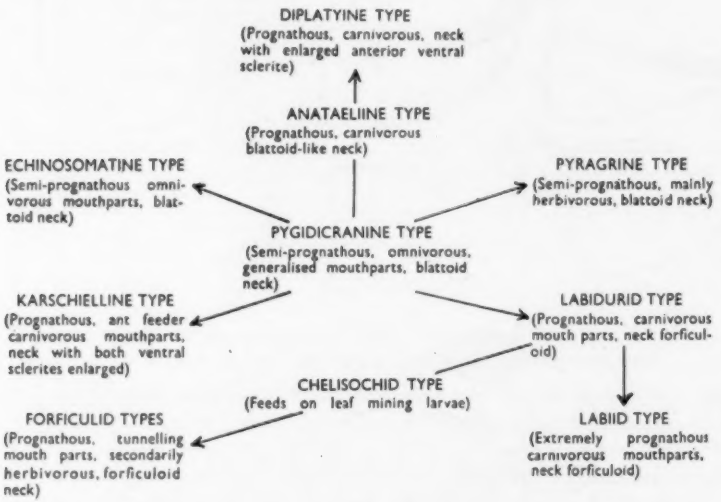


Fig. 28.—Forficulid types. Example—(A) *Doru lineare* (Eschscholtz). A. Dorsal view of head; B. Left mandible; C. Left lacinia; D. Left paraglossa. Examples (B) *Forficula auricularia* L. E. Left mandible; F. Left lacinia; G. Left paraglossa. Example (C) *Diaperasticus erythrocephalus* (Olivier). H. Dorsal view of head of male; J. Dorsal view of head of female showing tentorium, etc.; K. Left mandible; L. Left lacinia; M. Left paraglossa.

Diaperasticus (Fig. 28H and J) the reduction in the number of dorsal fibres of the mandibular adductor muscles has led to a reduction in the length of the epicranial sclerites as in the labiid type, but also to a reduction in the width of the sclerites, and the temporal sutures therefore run into the frontal sutures instead of the ocular sutures as in most other earwigs.

Of the feeding habits of the Forficulidae little is known. Xambeu (1903) states that the primitive forficulid, *Chelidura dilatata* Lafrenaye, feeds upon " tender vegetables, fruits, worms and larvae—and has pronounced carnivorous tastes ". The feeding habits of *Forficula auricularia* have been studied by many authors and their work reviewed by Crumb, Eide & Bonn (1941). Their opinion is that the insect is omnivorous with " a decidedly greater proportion of food derived from plant than from animal sources ". Calvert & Calvert (1917) records *Doru* sp. as occurring in maize fields, but little is known of its feeding habits or of *Diaperasticus*, though the crops of the few specimens available for study contained the remains of plant matter.

The relationship between the main types of head and neck may be summarised as follows :—


DIPLATYINE TYPE
(Prognathous, carnivorous, neck with enlarged anterior ventral sclerite)
ANATAELIINE TYPE
(Prognathous, carnivorous blattoid-like neck)
ECHINOSOMATINE TYPE
(Semi-prognathous omnivorous mouthparts, blattoid neck)
PYRAGRINE TYPE
(Semi-prognathous, mainly herbivorous, blattoid neck)
PYGIDICRANINE TYPE
(Semi-prognathous, omnivorous, generalised mouthparts, blattoid neck)
KARSCHIELLINE TYPE
(Prognathous, ant feeder carnivorous mouthparts, neck with both ventral sclerites enlarged)
LABIDURID TYPE
(Prognathous, carnivorous mouth parts, neck forficuloid)
CHELISOCHID TYPE
(Feeds on leaf mining larvae)
FORFICULID TYPES
(Prognathous, tunnelling mouth parts, secondarily herbivorous, forficuloid neck)
LABIID TYPE
(Extremely prognathous carnivorous mouthparts, neck forficuloid)

Brain size

Compared with *Locusta* and *Blatta*, the dermapteran head possesses relatively larger cerebral and suboesophageal ganglia. It is possible that a minimum number of neurons are required for the efficient functioning of the nervous system and small animals might, therefore, be expected to possess relatively larger ganglia on that account. However true that may be in general, for the Dermaptera this cannot be the entire explanation because, as Fig. 10 shows, increase of brain size is associated with increased prognathism, the smallest brains being found in the slightly prognathous pygidicranid type and the relatively largest

brain in the prognathous labiid types. Amongst Insecta the largest brains are to be found in Hymenoptera and Diptera. In these orders increase of brain size is correlated with the reduction in the thickness of the oesophagus as an adaptation to liquid feeding habits. There are many exceptions to this general statement, but similar trends may be observed in cephalopod Mollusca, where the elaboration of the nervous system is likewise associated with liquid feeding habits. It is perhaps, therefore, no accident that the most complex behaviour patterns of insects are to be found in members of the Hymenoptera and Diptera. The earwig head is, therefore, of special interest in as much as it shows a method of increasing brain size without the adoption of liquid feeding habits. In many Hymenoptera and Diptera the head is hypognathous or only slightly prognathous. The ventral region of the head capsule contains the mouthparts and associated musculature. Hence the only means of increasing the size of the brain and the sub-oesophageal ganglia is by a reduction in the diameter of the oesophagus, achieved by the adoption of liquid feeding. In Dermaptera, the change to prognathism has taken place in such a way as to cause the brain to be rotated backwards over the sub-oesophageal ganglion. The musculature of the mouthparts is situated on the ventral side of the head capsule. Once the prognathous condition has been attained, brain size can be increased by lengthening the head capsule, without any necessity for restricting the thickness of the oesophagus nor the adoption of liquid feeding habits. It is interesting to note that the Dermaptera show an elementary form of social behaviour, but apart from *Forficula auricularia* little is known of the social habits of the order and in view of the wide range of brain size a comparative study of the social habits of the order would be of special interest. Finally it is interesting to speculate what latent potentialities might be developed if the Dermaptera, like the Hymenoptera and Diptera adopted liquid feeding habits.

SUMMARY

1. The Common Earwig (*Forficula auricularia*) is omnivorous with a strong preference for plant material. The prognathous head bears flat horizontally articulating mandibles, distally bearing a pair of teeth, joined by a cutting edge to a proximal molar area (Fig. 1). The anterior cranial edge is folded round the mandibular condyles to form a strong grip and in this region the head is internally strengthened by a series of struts. (Fig. 1D). The dilated genae accommodate the mandibular muscles. The maxillae have specialised laciniae which distally bear a pair of chisel like teeth. (Fig. 4). The hypopharynx is a large fleshy lobe, ventrally supported by the paraglossae of the simple labium. The maxillae and labium lie in a longitudinal groove on the under side of the head between the genae.

2. During feeding the maxillae have a rotatory action, which is out of phase with mandibular movement (Fig. 6). While feeding, the head is raised from a hypognathous to a prognathous position while the mandibles cut a series of transverse incisions in the food material, particles of which are then freed by the laciniae and pulled onto the hypopharynx for salivation prior to mastication between the mandibular molar areas. Masticated food is moved into the

functional mouth by orally directed setae on the posterior median corners of the mandibles and by suction.

3. The mouthparts of *Forficula* can only function effectively when the head is raised, in contrast to the mouthparts of *Locusta* and many lepidopterous larvae which can only feed when the head is lowered.

4. Most mouthpart movements are due to direct muscle action, but maxillary abduction, depression and protraction, lacinial and galeal abduction as well as hypopharyngeal and paraglossal elevation are due to haemocoelic fluid pressure.

5. The cranial nervous system resembles that of the Orthoptera except that the ganglia are relatively larger and the brain is rotated dorso-posteriorly over the suboesophageal ganglion. The brain is therefore, both front to back and inverted.

6. Compared with the Orthoptera the head of *Forficula* is prognathous due to the elongation of the hypostomal region (Henson, 1950) and this is functionally associated with a lengthening of the mouthparts and of the tentorial body on which the adductor muscles of the maxillae originate. The morphological features are functionally associated with the rotatory action of the maxillae for the specialised feeding habits described above.

7. The neck is highly complex and allows the head a wide range of movement. The lateral cervical sclerites are greatly reduced and the head is mainly supported by a collar like structure. The cervical muscles and nerves are shown in Fig. 13.

8. The abdomen and its main muscles are described. The abdomen maintains a haemocoelic fluid pressure.

9. It is suggested the Dermaptera evolved from a hypognathous herbivorous pterygote stock which became partly carnivorous by capturing their prey between forwardly directed mandibular teeth and drawing it into the cibarium by maxillary retraction. Prey capture being facilitated by raising the head. The pygidicranine type (Fig. 17) in many ways seems to approximate to such an omnivorous ancestor. From this type the pyragrine type can be derived as a secondary return towards herbivorous feeding, while the diplatyine, karschielline and labidurid types represent independent trends towards predominately carnivorous feeding habits. The labiid type can be derived from the labidurid type as a semi-subterranean earwig feeding carnivorously in compact humus. The chelisochid type closely resembles the labidurid type and the habits of *Chelisoches morio* in feeding upon leaving mining hispid larvae shows how a carnivorous stock of earwigs might have acquired the tunnelling herbivorous feeding habits of the forficulid type, which is regarded as being secondarily herbivorous.

10. The form of the cerebral nervous system is discussed.

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THE GENERA OF ARENICOLIDAE (POLYCHAETA)

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(With 2 plates and 2 figures in the text)

CONTENTS

	Page
Introduction	301
History of the genera *Arenicola* and *Branchiomaldane*	302
The genus *Arenicolides* Mesnil	302
The new genus *Abarenicola*	307
Formal diagnoses of the family and genera	310
A key to the genera of the larger Arenicolidae	312
Summary	312
References	312
Explanation of the plates	313

INTRODUCTION

For nearly half a century the standard work on the Arenicolidae has been the monograph by Ashworth (1912 a). He described the family as composed of two genera: *Branchiomaldane* Langerhans with the single species *vincenti* and *Arenicola* Lamarck in which all of the remaining species are placed. Many relevant anatomical and systematic papers have appeared since the publication of Ashworth's monograph and a few years ago I began to collect materials for the revision of the family which recent additions to our knowledge have made necessary. I soon began to suspect that the forms then included in the genus *Arenicola* would more naturally be separated into three genera, and subsequent experience has steadily strengthened this opinion.

The family appears to be one in which the genera are more clearly defined than the species. Not all of the currently accepted species are satisfactory and our views on the choice of suitable criteria for recognising the species in this family—and especially on the importance of such numerical characters as the number of chaetigerous segments or of nephridia—may stand in need of modification. On the other hand, one finds that all of the forms generally included in *Arenicola* fall unequivocally into three groups, sharply characterised by many conspicuous and important features, and evidently representing natural taxonomic units of higher grade than species. An individual worm can be assigned without hesitation to one or other of the three even if its specific status is doubtful, and I am convinced that the recognition of the three groups as genera, by the revival of *Arenicolides* Mesnil and the erection of the new genus *Abarenicola*, is a useful preliminary to the consideration of the problems which arise when the worms are sorted out into species.

HISTORY OF THE GENERA *ARENICOLA* AND *BRANCHIOMALDANE*

Lamarck (1801) removed the Linnean species *marinus* from *Lumbricus* and erected for it the originally monotypic genus *Arenicola* of which he later (1818) gave the following diagnosis : " Corpus molle, longum, annulatum, cylindricum, postice nudum; setarum fasciculi biseriales in parte media anticaque. Branchiarum externarum arbusculae aut penicilli ad basim fasciculorum dorsalium. Os terminale nudum. Oculi nulli." Additional species were added from time to time, including two in which the appendages continue serially to the hindmost segments of the body, so that the words " postice nudum " in the diagnosis ceased to be appropriate.

The family Telethusae was formed by Savigny (1820) for the reception of the single genus *Arenicola*. The family was re-named Arénicoliens by Audouin and Edwards (1833) and Arenicolidae by Johnston (1835).

The following species were included in *Arenicola* by Ashworth (1912 a) : *marina* (L.), *loveni* Kinberg, *cristata* Stimpson, *glacialis* Murdoch, *pusilla* Quatrefages, *assimilis* Ehlers, *ecaudata* Johnston, *branchialis* Audouin and Edwards. Since that date Berkeley & Berkeley (1939) have added *glasselli*. It has been shown that Ashworth included the members of at least two species under the name *pusilla* (Wells, 1954 a ; see also Healy & Wells, 1959). On the other hand, *glacialis* appears to be a local form of *marina* (Wells, 1957). All of these forms have the specialised, achaetous tail—" postice nudum "—except for *ecaudata* and *branchialis*. The commonly encountered name *grubii* Claparède is a synonym of *branchialis*.

The genus and species *Branchiomaldane vincenti* were based by Langerhans (1881) on some small worms from Teneriffe. He placed the new genus beside *Arenicola* in the Telethusae but stated that the new form showed unmistakeable relationship with the Maldanidae. The anatomy of *Branchiomaldane vincenti* was described in detail by Ashworth (1912 b). Its close resemblance to a post-larval *A. ecaudata* has often been pointed out (e.g. Fauvel, 1899 a, Ashworth, 1912 a). Although it was at one time regarded as a species of *Arenicola* by Fauvel (1899 a, b) its original systematic status is now generally agreed.

THE GENUS *ARENICOLIDES* Mesnil

In a discussion of the affinities of *Branchiomaldane*, Mesnil wrote the following passage (Mesnil, 1898, p. 638, repeated in Mesnil, 1899, p. 326) :

" Les affinités de *Br. Vincenti* me paraissent être avec *A. branchialis* et *A. ecaudata*; et elle en est peut-être plus voisine, phylogénétiquement, que *A. marina*. N'y a-t-il pas là une raison théorique à ajouter aux raisons morphologiques qui me semblent militer en faveur de la scission du genre Arenicola en deux; l'un renfermant les espèces *avec un petit nombre de sétigères* (17–19) *et une longue partie caudale achète*, qui comprendrait *A. marina*. . . " Here follows a list of the other " caudate " species known at the time " . . . at à qui on conserverait le nom *Arenicola*; l'autre avec *A. branchialis* et *A. ecaudata*, espèces à *un grand nombre de sétigères, et sans partie caudale achète*, que l'on pourrait appeler *Arenicolides* ? La famille des Arenicoliens comprendrait ainsi trois genres : *Branchiomaldane*, *Arenicola*, *Arenicolides*, dont les caractères

distinctifs sont indiscutablement aussi importants que ceux que les annélidologues les plus autorisés ont habitude d'employer pour séparer leurs genres."

This suggestion brought an immediate rejoinder from Fauvel (1899 a). Neither *Branchiomaldane* nor *Arenicolides*, in his view at the time, should be separated from *Arenicola*. About the main distinguishing feature of Mesnil's *Arenicolides*, he wrote (p. 147):

" Le caractère, tiré du nombre des segments branchifères et de l'absence de queue achète et abranche, est tout-à-fait superficiel. La queue de l'*A. marina*, par exemple, est absolument l'homologue des segments postérieurs des *A. ecaudata*, *Grubii* et *Vincenti* et it ne faut voir là qu'une simple modification adaptative. Le nombre des segments qu'elle comporte est d'environ une trentaine sur un individu intact, si on y ajoute les 19 sétigères, on obtient un total de près de 50 segments correspondant assez bien avec les nombres fournis par les 3 espèces sans queue : 50 à 60, en moyenne, chez l'*A. ecaudata*: 40 à 50 pour l'*A. Vincenti* et 30 à 40 pour l'*A. Grubii*."

Fauvel considered that the differences between *Arenicola* and *Arenicolides* were no greater than those which separate certain of the species within these genera, and concluded (pp. 149–50):

" En résumé, on remarque que toutes les espèces présentent un grand nombre de caractères communs, tandis que les différences qui les séparent, sont peu nombreuses et purement spécifiques. Si on attribuait a ces différences une valeur générique, il faudrait alors créer un genre pour chaque espèce. . . Placé entre l'alternative d'admettre un seul genre homogène ou d'en créer un distinct pour chaque espèce, il me semble que l'hésitation n'est pas possible. . . . Je propose donc la suppression des genres inutiles *Branchiomaldane* et *Arenicolides* pour revenir au genre unique *Arenicola* renfermant toutes les espèces connues d'Arénicoliens."

Subsequent authors, including Fauvel himself, have recognised the validity of *Branchiomaldane*. Nobody since Mesnil has suggested that *Arenicolides* deserves recognition as a separate genus, although several authorities have separated the species of *Arenicola* into two contrasted groups.

Gamble & Ashworth (1900) divided *Arenicola* into a caudate and an ecaudate section, corresponding to Mesnil's genera. They were followed in this course by Ashworth (1912 a), who enumerated an impressive list of differences between the two sections (pp. 83–5). His reasons for not recognising them as genera may be given in his own words (pp. 31–2):

" The writer, while not prepared to go so far as Prof. Mesnil suggests, admits that there is something to be said in favour of the recognition of the differences between the caudate and ecaudate species. The latter differ from the former in their brain and prostomium, in the absence of tail, and in the mode of branching of their gills, but the two ecaudate species diverge very sharply from each other in the number of their nephridia and the nature of their reproductive organs; in regard to the last-named character especially the differences between *A. branchialis* and *A. ecaudata* are as great as between the latter and any caudate species. The opinion of the writer is that the caudate and ecaudate species present such a number of common characters as to warrant their inclusion in a single genus, and that the differences presented

by the ecaudate species would be sufficiently recognised by regarding them as of sub-generic importance."

An unusual procedure was adopted by McIntosh (1915) in arranging the British species of *Arenicola*. He divided the family into two, but not the genus. His "Sub-Family I.—Arenicolidae caudata" housed *Arenicola marina* while *Arenicola ecaudata* and *A. branchialis* were placed in "Sub-Family II.—Arenicolidae ecaudata".

There is no formal recognition of the two sections in Fauvel (1927) although the presence or absence of an achaetous tail is the first distinction in his key to the species.

My own conviction is that Mesnil was right. The characters which separate the tailed from the tailless forms are more than sufficient to warrant generic distinction. In making this assertion it is not only in judgment that I differ from the authorities who rejected his opinion, for in fact the distinguishing characters are more numerous, and in some ways more profound, than they knew.

The following points may be noted.

(1) The tail is a region of the body specialised in more than one way (Wells, 1950). The segments of an *ecaudata* or a *branchialis* are laid down by a pygidial growth zone which forms about 42 segments in *branchialis* and about 64 in *ecaudata* and then stops (Ashworth, 1912 a, p. 36). These are the numbers of segments in the whole body. After this there are generally losses from the hind end, many full-grown specimens having lost one quarter or one third of their original number of segments, but there is no regrowth. In a caudate species, the pygidial growth zone lays down a great number of very short "reserve segments" at the base of the tail; these gradually lengthen, the hinder ones growing first, so the result is a continual backwards growth of tail which makes up for the very considerable losses from the hind end which occur by accident or autotomy (Pl. I, fig. 2). The number of segments may be greater than was estimated by Fauvel in the passage cited above. The maximum for the tail of *marina* is about 60 or 70 segments; as many as 175 have been counted in the tail of *loveni* (Ashworth, 1912 a, p. 38).

A body segment of *ecaudata* or *branchialis* (except for three or four at each end of the body) consists of five annuli, of which the last but one, the chaetigerous annulus, is larger than the others and bears the appendages. A trunk segment of any of the caudate species has the same structure, but the tail segments lack appendages and their annuli increase steadily in number as they grow. A segment from the base of the tail may have only one or two annuli; at the tip there may be as many as 25 annuli to a segment (Wells, 1950, p. 39).

Clearly, the evolution of an achaetous tail was by no means a simple modification since it involved both the structural organisation and the growth pattern of the hinder part of the body. We can hardly agree with Fauvel that this character is "tout-à-fait superficiel".

(2) The prostomium is differently formed in the caudate and ecaudate species (Ashworth, 1912 a, figs. 1–4; Wells, 1950, figs. 9–12 and 15–19). That of *marina* is a triangular area with the position of the roughly X-shaped brain

marked by shallow grooves on its dorsal and anterior faces. The same pattern is seen in all of the caudate species, although in those to be separated as *Abarenicola* in the following section the prostomium extends farther laterally and in some of them its lateral lobes are enlarged as the statocysts are lost. In *ecaudata* and *branchialis*, on the other hand, the prostomium is a flat strip running transversely across the dorsal and lateral aspects of the head, and sometimes hard to distinguish from the general body surface ; the brain is comparatively large and extends laterally much farther than in the caudate forms. Anterior to the brain, and often overhanging the mouth, the ecaudate species have a roughly conical projection which corresponds to the " upper lip " of the caudate forms and which has sometimes been confused with the true prostomium. (Pl. 2, fig. 8).

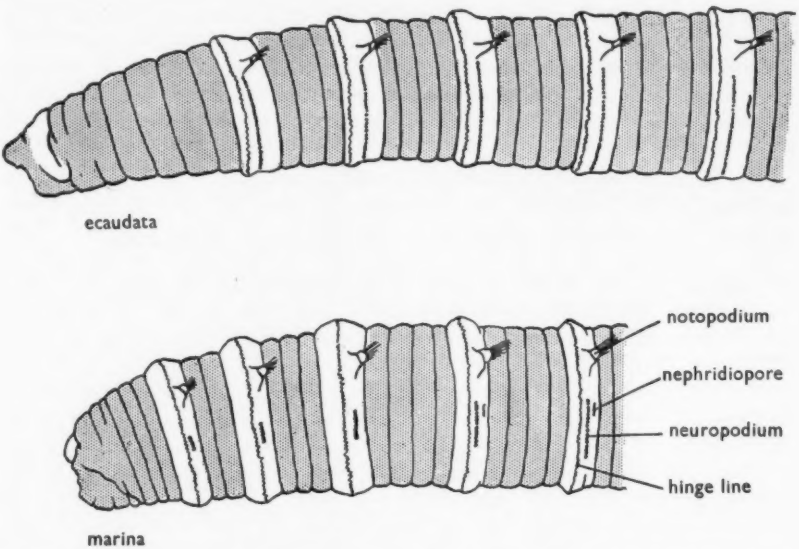

Fig. 1.—Lateral views of the anterior ends of *Arenicolides ecaudata* and *Arenicola marina*. The chaetigerous annuli and prostomia are shown white.

(3) The neuropodia show a striking distinction (Fig. 1). A neuropodium is seen externally as a dorsi-ventrally directed cleft from which the tips of a single row of stout, sigmoid chaetae project. In *marina*, as in all species of *Arenicola* in my restricted sense, the neuropodia of the branchiate segments, run from the immediate neighbourhood of the mid-ventral line nearly to the level of the notopodia ; those of the more anterior segments are shorter and their ventral ends move away from the mid-ventral line as the anterior end is approached (Pl. I, fig. 1). Basically the same pattern is seen in *Abarenicola*, the most important departure being that here the ventral ends of the neuropodia of the branchiate segments remain fairly remote from the mid-ventral

line. In the ecaudate species, on the other hand, the neuropodia are long and approach the mid-ventral line in all segments ; those of the most anterior segments are the longest, and extend up to (*ecaudata*) or even above (*branchialis*) the level of the notopodia (Fig. 1).

This difference was apparently not noticed by earlier writers. According to Ashworth (1912 a, p. 35), in all of the species included by him in *Arenicola* " The neuropodia of the anterior segments (the first six or seven) are smaller than the rest "—although his illustrator, A. K. Maxwell, drew the neuropodia of the two ecaudate species correctly (his Pl. II). Fauvel (1927, Fig. 58 a) even drew the neuropodia of *branchialis* as shorter in the anterior segments, after the manner of *marina*.

(4) The most anterior gill (which may be reduced or absent) is nearly always on segment vii in the caudate species. In the ecaudate species it is five or more segments farther back. Moreover, the two groups of species differ markedly in the form of the gills, as shown by Ashworth (1912 a, pp. 56–61, 83, 85). The main stems " radiate, in the ecaudate species, from a common basal trunk, or, in the caudate species, from a crescentic fold ", and they bear branches unilaterally in the ecaudate species and bilaterally in the caudate ones.

(5) There are important differences in the proboscis and associated internal structures (Wells, 1952, 1954 b). The extruded proboscis of a caudate worm shows a buccal mass, whose papillae have the form of blunt pegs or conical teeth, and a rounded pharynx with numerous, fine papillae. In *ecaudata* or *branchialis* the form of the extruded proboscis is more like that of a loud-speaker cone, the apex being towards the mouth ; the papillae on the buccal mass differ strikingly from those of the caudate species in being sucker-shaped (Pl. 2, figs. 7, 8). The proboscis of *ecaudata* or *branchialis* is driven out largely by the contraction of a very powerful gular membrane, prolonged backwards into two enormous septal pouches. These structures alone can bring about full extrusion of the proboscis, even when the possibility of a rise in the general coelomic pressure has been experimentally excluded (Wells, 1954 b). In the caudate species the gular membrane and septal pouches vary greatly in strength, but, with the possible exception of *loveni*, they are never as powerfully built as in the ecaudate ones. The characteristic " buccal membrane " of *ecaudata* and *branchialis*, absent in the caudate species, is perhaps connected with the difference in form of the buccal papillae.

(6) On dissecting one of the caudate species, it will be noticed that the fibres of the longitudinal muscle layer are grouped into a great number of longitudinal columns, which branch and anastomose with their neighbours and form a conspicuous and characteristic background to the dissection (Pl. 2, figs. 5, 6). In the ecaudate forms, on the other hand, the body wall presents a smooth surface internally ; the longitudinal layer is covered by a continuous peritoneal sheet which is only occasionally grooved to accommodate a blood vessel (Ashworth, 1912 a, Pls. 4, 8, 9 ; Wells, 1950, Fig. 3).

(7) The nephrostome is a flattened funnel with two lips, generally described as dorsal and ventral. The dorsal lip bears a row of ciliated vascular processes.

In the ecaudate species the dorsal lip processes are digitiform, and often bifid or trifid ; the ventral lip is deeply notched in the middle. In the caudate species, the dorsal lip processes are flattened ; the ventral lip margin is entire, though it may be thrown into more-or-less conspicuous folds or frills (Ashworth, 1912 a, pp. 71, 83, 85).

The above are clearly-definable characters, but they are only aspects of a pervading " differentness " that becomes more and more impressive as one gets to know the worms. As anyone familiar with the living animals will understand, the ecaudate species are more slender and agile than the caudate, and I question whether the vernacular term " lugworm ", meaning a clumsy worm, is properly applied, as has sometimes been done, to a member of the ecaudate group.

The habits of the caudate species appear in general to resemble those of *Arenicola marina*, described in detail by Wells (1945). They typically inhabit beaches of sand or muddy sand, ingesting the substrate (usually with a high proportion of surface material) and in most cases producing the well-known, cylindrical castings. The ecaudate species are found in sandy or muddy material among stones or in clefts between rocks (Ashworth, 1912 a; Fauvel, 1927). To some extent the anatomical differences between the two groups of species could be interpreted as adjustments to their ways of life ; for example, it has been suggested that an achaetous tail, containing no vital organs and renewed by growth from the base, is an adaptation to life on exposed sand flats (Wells, 1950, p. 40). But the fact remains that the differences are numerous, affecting many aspects of the worms' organisation, and many of them are more profound than one would expect to encounter between members of the same genus.

Some of the species are distinguished by striking characters ; for example, *ecaudata* differs from all other Arenicolidae, including *branchialis*, in having 13 pairs of nephridia instead of the usual 5, 6 or 7, and in a curious modification of the gonads which become enlarged and " form the most striking feature in a dissection of this species " (Ashworth, 1912 a, pp. 71–3). But at all other points of their organisation the differences between *ecaudata* and *branchialis* are comparatively trivial. There is no case in which the differences between two species of one of the genera, as here defined, are comparable in number and aggregate importance to those which separate the genera.

THE NEW GENUS *ABARENICOLA*

According to Ashworth (1912 a, pp. 157–8) : " The caudate species are divisable into two divergent series, one—including *A. marina*, *glacialis*, *loveni* and *cristata*—possessing elongate neuropodia, septal pouches and a single pair of oesophageal glands, the other—comprising *A. assimilis* and *pusilla*—in which the neuropodia are short, septal pouches are absent, and there are several pairs of oesophageal caeca ". No formal proposal has yet been made to recognise these " divergent series " as distinct genera. It seems to me, however, that the differences are profound enough to justify generic separation, particularly as the resulting groups of species differ among themselves only in characters which are clearly of less importance. I therefore propose to restrict *Arenicola* Lamarck to the group of species typified by *A. marina* and including

A. glasselli Berkeley besides those enumerated above, and to set up the genus *Abarenicola* for the other. The species *claparedii*, *pacifica* and *vagabunda*, subsequently split off from *pusilla* as defined by Ashworth, belong of course to *Abarenicola*. It is a curious fact that the characters which are useful for separating the species are of different nature in the two genera, as if each genus had the potentiality to radiate in its own particular way.

The following distinctions between *Arenicola* and *Abarenicola* may be noted.

(1) At the hinder end of the oesophagus in *Arenicola* (as also in *Arenicolides*) there is a single pair of oesophageal glands or caeca (Pl. 2, fig. 5). These structures are generally more-or-less conical, though their shape varies somewhat with their functional condition. They are internally laminated, and communicate with the hinder end of the oesophagus through relatively narrow ducts. They presumably serve to produce a digestive secretion. Instead of this single pair of glands, the oesophagus in *Abarenicola* has a longitudinal row of caeca on each side (Pl. 2, fig. 6 ; Healy & Wells, 1959, Pl. 2). The number, on each side of the oesophagus, may be as low as 4 or as high as 20 ; it depends on heredity rather than on age and can be used as a taxonomic character (Healy & Wells, 1959). The most anterior caecum on each side is considerably longer than the rest, and usually has thinner walls. The shorter ones which follow it generally show a grading in size, the posterior one being the smallest of all. These caeca have wide openings into the hinder end of the oesophagus, and it is occasionally found, in preserved worms, that one of the long anterior pair is invaginated into the gut lumen. It will be seen that the caeca of *Abarenicola* represent, not merely a multiplication of structures otherwise like those of *Arenicola*, but a reorganisation of the apparatus.

(2) The mechanism of proboscis movement is apparently different in the two genera. In the fully extruded proboscis of *Abarenicola*, but not of *Arenicola*, a fine " waist-line " can be seen running round the pharynx ; this is due to a difference in the way in which the retractor muscle sheath is attached to the pharynx (Wells, 1952, Fig. 12 and Pl. 1). In *Arenicola*, the gular membrane is muscular and has a pair of septal pouches, varying in size from *loveni*, in which they are enormous, to *marina* in which they are small (Ashworth, 1912 a, Pl IV). Even in *marina*, however, the buccal mass can be everted by contraction of the gular membrane, unassisted by fluid pressure from behind (Wells, 1954 b). The gular membrane in *Abarenicola* is so thin and delicate that it cannot possibly play any part in proboscis extrusion; it has no septal pouches.

(3) In *Arenicola*, the prostomium is a small, roughly triangular structure which can be wholly retracted into the nuchal pouch. An area of ordinary body wall intervenes between its antero-lateral angles and the statocysts (Ashworth, 1912 a, Fig. 1 ; Wells, 1950, Fig. 9). In *Abarenicola*, the prostomium has the same essential form but its antero-lateral corners extend sideways to the position which the statocysts occupy if present (they are lacking in some of the species. The prostomium is therefore broad in front, and it cannot be retracted into the nuchal pouch (Ashworth, 1912 a, Figs. 2, 4 ; Wells, 1950, Fig. 15).

(4) In *Arenicola*, the neuropodia of the branchiate segments extend ventrally nearly to the mid-ventral line. In *Abarenicola* this is not so ; the

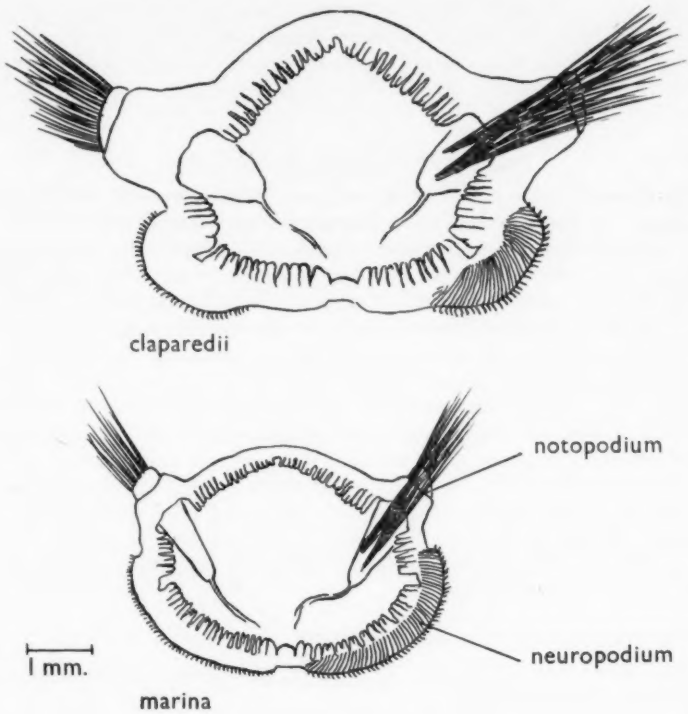

Fig. 2.—Drawings of chaetigerous annulus xvi, as seen in transected specimens of *Abarenicola claparedii* and *Arenicola marina*. The gills are omitted. The full extent of the chaetae is shown on the right, the projecting portions only on the left.

ventral ends of the neuropodia of a branchiate segment are separated by a distance roughly equal to the length of a neuropodium (Fig. 2 ; Pl. 1, figs. 3 and 4). This is the meaning of " elongate neuropodia " and " short neuropodia " in the citation from Ashworth at the beginning of the present section.

The differences between *Arenicola* and *Abarenicola* are clearly considerable, but they do not appear to be as great, in the aggregate, as those which separate *Arenicolides* from the two caudate genera. *Branchiomaldane* is " most nearly related to the ecaudate species " (Ashworth, 1912 a, pp. 156, 159), and it may be that the taxonomists of the future will revive the forgotten suggestion of McIntosh (1915), quoted on an earlier page, by dividing the Arenicolidae into two subfamilies, for the caudate and ecaudate forms respectively.

FORMAL DIAGNOSES OF THE FAMILY AND GENERA

The diagnoses of the genera are followed by lists of the currently recognised species which constitute the genus. The reference of any form to its genus is unequivocal, but it seems likely that certain of the species will in future be split, or merged within the genera. I have chosen, as types of *Arenicolides* and *Abarenicola*, species whose future seems reasonably secure, and whose anatomy has been investigated in detail (Gamble & Ashworth, 1900 ; Wells, 1950, 1952, 1958).

Family ARENICOLIDAE Johnston 1835

Polychaeta of elongate, cylindrical form. The achaetous anterior region, or " head ", is composed of a prostomium without tentacles or palps, a peristomium without cirri, and another achaetous segment. The setigers follow, each divided into secondary annuli, one of which is the chaetigerous annulus and bears on each side a notopodium with capillary chaetae and a neuropodium with a single row of crotchets*. In some species the number of setigers is restricted, usually to 19, and the remaining segments are specialised to form an achaetous " tail ". Gills are borne, in the adult, on a number of successive setigers. The eversible proboscis is jawless. Glandular caeca, one pair or more, are present on the posterior part of the oesophagus. There is a single pair of hearts, at the sides of the junction of oesophagus and stomach. Coelomic septa have disappeared from many consecutive segments in the middle region of the body but are present at the anterior border of the first, third and fourth setigers, and also to a greater or less extent in the intestinal region. In many species, the first septum bears a pair of backwardly projecting septal pouches.† Nerve cord not ganglionated.

Type genus : *Arenicola* Lamarck 1801.

The family also includes : *Abarenicola* gen. nov., *Arenicolides* Mesnil, *Branchiomaldane* Langerhans.

ARENICOLA Lamarck 1801

Arenicolidae with an achaetous tail. Prostomium small, retractile, in the form of a triangle with the rounded base anterior; with a shallow groove marking the attachment of the brain. Statocysts present, with or without a tube to the exterior. Setigers (except the first two or three) subdivided into five annuli. Gills branched, the first (which may be reduced or absent) on setiger vii. The neuropodia of the hinder branchiate segments, but not those of the more anterior segments, approach close to the mid-ventral line. One pair of oesophageal caeca, opening by separate ducts. Septal pouches present.

* In certain segments the parapodium also gives rise to muscular specialisations of the general surface of the chaetigerous annulus (Wells, 1944).

† I have suggested (Wells, 1952) that the septum at the anterior boundary of the first setiger gives rise both to the retractor muscle sheath and to the gular membrane, or " first diaphragm " of previous authors. The septal pouches are part of the gular membrane and their cavity is an extension of the space between gular membrane and retractor sheath. According to my view, this space is not coelomic.

Nephridia five to seven pairs, the first opening on setiger iv or v. Dioecious ; gonads on the nephridia.

Type species : *Lumbricus marinus* Linnaeus 1758.

The originally monotypic genus now includes : *cristata* Stimpson 1856 ; *loveni* Kinberg, 1866 ; *glacialis* Murdoch, 1885—which is probably a form of *marina* (see Wells, 1957) ; *glasselli* Berkeley and Berkeley 1939, which may be a form of *cristata* (see Fauvel, 1950).

ABARENICOLA gen. nov.

Arenicolidae with an achaetous tail. Prostomium non-retractile, in the form of a triangle with lateral extensions of its (anterior) base ; with a shallow groove marking the attachment of the brain. Statocysts either present, with a tube to the exterior, or absent. Setigers (except the first two or three) subdivided into five annuli. Gills branched, the first (which may be reduced or absent) on setiger vii or viii. None of the neuropodia approaches close to the mid-ventral line. Oesophageal caeca more than one pair. Gular membrane very thin; septal pouches absent. Nephridia five or six pairs, the first opening on setiger iv or v. Dioecious ; gonads on the nephridia.

Type species : *Arenicola claparedii* Levinsen 1883.

The genus also includes *pusilla* (Quatrefages, 1865), which has sometimes been regarded as identical with *claparedii* but is in fact distinct (Wells, 1954 a), *pacifica* Healy & Wells, 1959, *vagabunda* Healy & Wells, 1959, and *assimilis* (Ehlers, 1897). Work now in progress suggests that the last-named should be split into two or more species.

ARENICOLIDES Mesnil 1898.

Arenicolidae without an achaetous tail. Prostomium a non-retractile, transverse band ; brain large, transversely elongated. Statocysts present, without a tube leading to the exterior. Setigers (except the first two or three) subdivided into five annuli. Gills branched, the first (which may be reduced or absent) on setiger xii or xvi. All the the neuropodia approach close to the mid-ventral line. One pair of oesophageal caeca, opening by separate ducts. Septal pouches present. Nephridia 5 or 13 pairs, the first opening on setiger xv. Dioecious ; gonads on the nephridia.

Type species now designated: *Arenicola ecaudata* Johnston 1835. The genus also includes *branchialis* (Audouin & Edwards 1833), of which *grubii* (Claparède 1868) is a synonym still in occasional use.

BRANCHIOMALDANE Langerhans 1861

Small, lightly-pigmented Arenicolidae without an achaetous tail. Prostomium bluntly conical, as in post-larval forms of other genera. Statocysts absent. Gills consisting of 1 to 4 simple filaments, the first on setiger xviii or farther back. Branchiate segments subdivided into two annuli. None of the

neuropodia approach close to the mid-ventral line. One pair of oesophageal caeca, opening by a common duct. Septal pouches absent. Nephridia two pairs, opening on setigers v and vi. Hermaphrodite ; gonads on the septa or oblique muscles.

Type species : *vincenti* Langerhans 1881.

No other species has been named.

A KEY TO THE GENERA OF THE LARGER ARENICOLIDAE

The genera defined above are sharply delimited and there should be no difficulty in assigning any individual specimen to its genus. The following key shows the characters that will be found most useful when working on preserved material of the larger forms. The work of Ashworth (1912 a) may be consulted for *Branchiomaldane* and the post-larvae of the others. I am now engaged on a revision of the species and believe that it will be necessary to make adjustments to the current system ; the inclusion at the present stage of a key to the species would therefore be premature.

1.	Differentiated achaetous tail present; first gill on setiger vii or viii; the neuropodia of the most anterior setigers are short and their ventral ends do not closely approach the mid-ventral line (Fig. 1, p. 305)	2.
–.	No differentiated tail; first gill on setigers xii or xvi; the neuropodia of the most anterior setigers are long and closely approach the mid-ventral line (Fig. 1, p. 305)	genus *Arenicolides*
2. (1)	One pair of oesophageal caeca (Pl. 2, fig. 5) ; neuropodia of the hinder branchiate segments closely approach the mid-ventral line (Pl. 1, fig. 3)	genus *Arenicola*
–.	More than one pair of oesophageal caeca (Pl. 2, fig. 6) ; neuropodia of the hinder branchiate segments do not closely approach the mid-ventral line (Pl. 1, fig. 4)	genus *Abarenicola*

SUMMARY

It is argued that the species currently grouped as *Arenicola* Lamarck should be distributed between *Arenicola*, *Arenicolides* Mesnil and *Abarenicola* gen. nov.

Formal diagnoses of the family and its constituent genera are given, and suggestions are made as to the most convenient characters for referring arenicolid worms to their genera.

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EXPLANATION OF THE PLATES

PLATE 1

Fig. 1.—Side view of the anterior end of an *Arenicola marina* from Thorpe Bay, Essex. Compare Fig. 1, p. 305.

Fig. 2.—Dorsal halves of the horizontally-bisected base and tip of the tail of an *Arenicola marina* from Thorpe Bay. Note the enormous lengthening of the segments at the tip.

Figs. 3, 4.—Ventral views of the hinder branchiate segments and base of the tail of an *Arenicola marina* from Thorpe Bay (3) and an *Abarenicola claparedii* from Naples (4). The neuropodia are seen as white lines ; they approach closely to the mid-ventral plane in *Arenicola* but not in *Abarenicola* (compare Fig. 2, p. 309).
All photographs in Pl. 1 are by the late Mr F. J. Pittock.

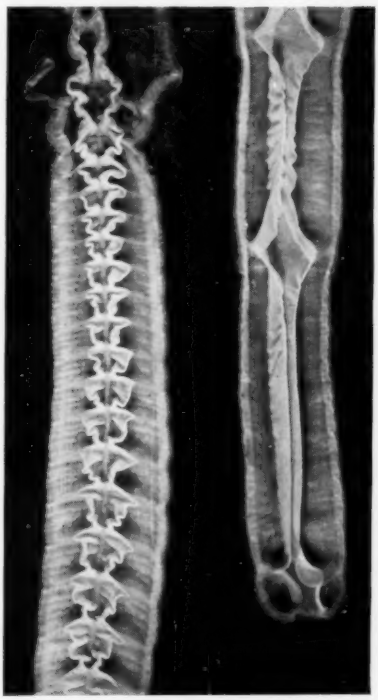
PLATE 2

Figs. 5, 6.—Dissections of an *Arenicola marina* from Plymouth (5) and an *Abarenicola assimilis* from Christchurch, New Zealand (6). Note the oesophageal caeca just anterior to the very dark hearts. In *Arenicola* there is one pair of caeca. In *Abarenicola* there are more ; the worm in the photograph has a large pair (the one on the left happens to be distended and is pinned aside) then six small caeca on each side. Photographs by Mr A. Veenstra.

„ 7, 8.—Lateral views of two specimens in which the buccal mass, but not the pharynx, is extruded. Note the tooth-shaped buccal papillae of *Arenicola marina* (7), the sucker-shaped buccal papillae of *Arenicolides ecaudata* (8), and the broad prostomium of the latter (compare Fig. 1, p. 305). Both worms are from Plymouth. Photographs by Mr J. Armstrong.

1. *marina.*

5 mm.



2. *marina.*

5 mm.

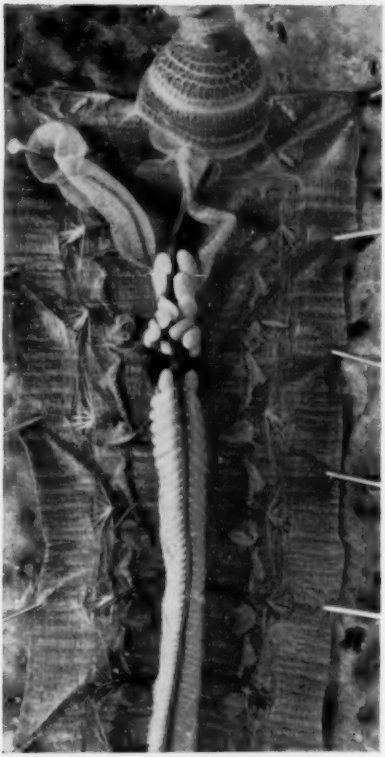
3. *marina.*

5 mm.

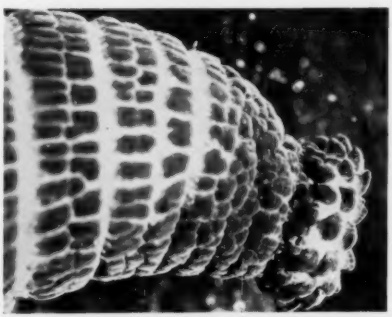
4. *claparedii.*

5 mm.

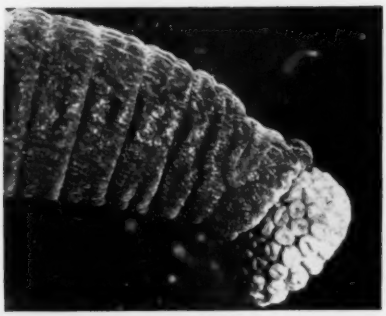
5. *marina.* 1 cm.



6. *assimilis.* 1 cm.



7. *marina.* 5 mm.



8. *ecaudata.* 5 mm.

THREE NEW LUGWORMS (ARENICOLIDAE, POLYCHAETA) FROM THE NORTH PACIFIC AREA

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(With 4 plates and 3 figures in the text)

CONTENTS

	Page
Introduction	315
Anatomical characters	316
Geographical distribution	319
Biology of *pacifica* and *vagabunda*	321
Distribution in False Bay	321
Forms of the burrows	324
Commensal pea crabs	325
Breeding season	326
Spawning behaviour, "Surfacing" of *vagabunda*	327
Discussion	329
Definitions	329
Acknowledgments	331
Summary	331
Appendix	331
References	333
Explanation of the plates	334

INTRODUCTION

The Arenicolidae of the North Pacific area are currently referred to three species, each of which occupies a distinct geographical range. The warm-water *Arenicola cristata* Stimpson extends southwards from Japan and California. The more northerly *Arenicola marina* (L.), represented by *A. m. schantarica* Zachs in the Sea of Okhotsk and *A. m. glacialis* Murdoch from Point Barrow and Bering Strait, is apparently restricted to the coldest waters, although the existence of a single specimen of *A. m. marina*, almost certainly collected from deep water near Vancouver, suggests that the species extends further into the area than has been supposed (Wells, 1957). The following paper is concerned with the third group of worms, which occupies a wide arc along the coasts from Siberia and Japan through the Kurile Islands, Alaska, British Columbia, Washington, and Oregon to California. These worms have hitherto been

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recorded as *Arenicola claparedii* Levinsen, or occasionally as *A. pusilla* Quatrefages, with which Levinsen's species was for a time confused. According to a revision of the genera of Arenicolidae, published in front of this paper, they belong to the new genus *Abarenicola* (Wells, 1959 b).

Naturalists working on the shores around Seattle and Vancouver have long known that their region contains two kinds of lugworm, living in separate colonies and differing at least in appearance, although they both conform to the currently accepted diagnosis of *Abarenicola claparedii*. It has been locally rumoured, though never published, that they deserve recognition as distinct species. We have studied this problem in False Bay, San Juan Island, where the two forms occur side by side. The following paper reports on our results, and also on the examination of a number of specimens hitherto referred to Levinsen's species and collected from localities dispersed over the entire North Pacific range. These were compared with examples from Naples, the locality from which Levinsen's original material was taken. We conclude that the North Pacific "*claparedii*" consists of three forms, distinct from each other and from the original Neapolitan one.

The various forms are distinguished by characters which have not hitherto been regarded as of taxonomic importance in the Arenicolidae. It is essential, when identifying preserved material, to open the body cavity so that the number of caeca at the hinder end of the oesophagus may be determined. The superficial characters of the nephridiopores are also of importance. We have found it easier to discuss than to decide whether the Pacific forms should be described as new species or as subspecies of *A. claparedii* and it may well be that our conclusions will have to be modified when further evidence becomes available.

Throughout the descriptive sections of our paper, the Pacific forms are referred to as *pacifica*, *vagabunda* and *oceanica*, the name *claparedii* being reserved for the European one. The status and relationships of these forms are considered in the Discussion.

ANATOMICAL CHARACTERS

The following characters are common to *claparedii*, *pacifica*, *vagabunda* and *oceanica*. The body consists of an achaetous head, comprising the prostomium and two fused body segments, followed by a trunk of 19 chaetigerous segments and an achaetous tail. The most anterior gill is typically on trunk segment vii, but this gill may be reduced in size or even absent. The neuropodia of the branchiate segments are separated ventrally by a wide interval, about equal to the length of a neuropodium (Wells, 1959, text-fig. 2). The three most anterior chaetigerous annuli are very powerfully developed and the ordinary annuli between them are reduced in number and extent (Wells, 1950, fig. 4 and p. 23). There are five pairs of nephridia, opening on trunk segments v to ix. Septal pouches are absent, and there are more than one pair of glandular caeca at the hinder end of the oesophagus (Pl. 2). Statocysts are absent, and the prostomium is expanded into two large lateral lobes ; these lie in front of the wide "otic grooves" which replace the statocysts (Wells, 1950, figs. 15–17).

The differences between *claparedii*, *pacifica*, *vagabunda* and *oceanica* include characters which have not previously been regarded as of taxonomic importance. The most useful in practice are, first, the number of glandular caeca on the posterior part of the oesophagus and, second, the external structure of the nephridiopores.

Near its junction with the stomach, the oesophagus bears several laterally placed, glandular caeca, of which the most anterior on each side is much longer than the rest (Pl. 2). In exceptional cases, the anterior caecum is invisible when the worm is first opened, having invaginated itself into the lumen of the oesophagus. Previous writers have often described the worms as having so-many " pairs " of oesophageal glands or oesophageal caeca. However, the structures are not strictly paired and may differ in number on two sides of the oesophagus. Our practice is to give the count as it would be taken on one side of the oesophagus, using the formula $1 + n$ where 1 stands for the long caecum and n is the number of small ones. A worm in which the number of small caeca is different on the two sides, such as the *vagabunda* in Pl. 2, is recorded in the form $1 + n/m$.

Table 1 shows the values for the four forms ; it may be added that the great majority of *pacifica* are $1 + 4$ or $1 + 5$. Table 2, based on a series of worms collected in False Bay in 1954, shows that the number of caeca does not vary significantly with size, and is therefore a reliable indicator of the particular form.

TABLE 1

Form	Oesophageal caeca	Nephridiopores
claparedii	1+3 or 1+4	hooded
pacifica	1+3 to 1+6	naked
oceanica	1+7 to 1+9	hooded
vagabunda	1+11 to 1+18	hooded

TABLE 2

The average numbers of oesophageal caeca in different length groups. The length is measured in mm. from the mouth to the junction of trunk and tail.

	pacifica		*vagabunda*	
Length group mm.	Number of worms	Number of caeca	Number of worms	Number of caeca
30/59	27	1 + 4·05	13	1 + 15·05
60/89	27	1 + 4·10	23	1 + 14·75
90/119	8	1 + 4·05	22	1 + 14·80
>119	—	—	5	1 + 15·40

In most species of Arenicolidae, such as *Arenicola marina*, the nephridiopore is seen externally as a plain, dorsi-ventrally elongated slit between more-or-less conspicuous, tumid lips. Such a nephridiopore will be described as " naked ". The alternative type, " hooded ", is seen in *vagabunda*, in which form the primitive nephridiopore is concealed by the growth of an outer hood, consisting of two lips separated by an antero-posteriorly elongated cleft. The ventral

lip is larger than the dorsal one. If the hood is stretched, or cut away, the primitive, dorsi-ventrally elongated slit can be seen (Plate 3).

The nephridiopore of *claparedii* has a delicate hood, sometimes so thin as to be transparent, but essentially like that of *vagabunda*.

In *pacifica* the nephridiopores are naked, but many specimens show a more-or-less well-developed rudiment or vestige of the hood, especially on the ventral side of the nephridiopore. We have seen particularly conspicuous vestigial hoods in a batch of material from Departure Bay, Nanaimo, B.C., but even in this case there would be little likelihood of confusion with a fully hooded nephridiopore.

In *oceanica* the nephridiopores are hooded but the structural details are rather variable. Specimens from the Aleutian Islands closely resemble *vagabunda* in their nephridiopores. Those from the Pacific beaches of Vancouver Island and U.S.A. have well-developed ventral hoods, but their dorsal lips are somewhat small and receding, and may be apparently absent.

There is generally, though not invariably, a colour difference between *pacifica* and *vagabunda*. These are the two forms occurring in False Bay, where we have had many opportunities of comparing the living worms. Most living specimens of *pacifica* are pale pinkish orange, sometimes with a diffuse greenish brown darkening of the anterior half-dozen segments. Preserved specimens are generally a rather gingery brown. The colour of *vagabunda* is typically a very dark, blackish brown ; as in other darkly coloured Arenicolidae the worms show conspicuous green colour when immersed in sea water. However, pale *vagabunda* and dark *pacifica* are sometimes encountered.

The Neapolitan worms are pale in colour and comparatively small—up to 60 or 70 mm. in length, and generally less. We have not seen *oceanica* alive ; the preserved specimens of this form are brown or black and they include the largest worms encountered during the present study. We have examined, and determined as *oceanica*, the specimens of which Ashworth wrote (1912, p. 121) : " Those from Dutch Harbour, Unalaska, are the largest known examples of the species ; their length, 160 mm., is not remarkable, but they are of very massive build, being 50 to 60 mm. in girth at the widest point ".

The muscular development of the body wall is strikingly different in *pacifica* and *vagabunda*. On cutting open the living or recently killed worms, the greater resistance to the scissors of the body wall in *pacifica* is very noticeable, and this form is also firmer in " feel " when handled. If the worms are narcotised in isotonic $MgCl_2$, then killed in Mg-formalin, *pacifica* always assumes the characteristic " death attitude " shown in Plate 1, in which the anterior segments are narrowed and arched, and their chaetigerous annuli are prominently raised. This attitude is never shown by *vagabunda* and may be attributed to a difference in the development of the circular muscle layer of the body wall.

It was pointed out by Gamble & Ashworth (1900) that the nerve cord of *claparedii* remains through life in its original, larval position in intimate contact with the epidermis. In most species of Arenicolidae the cord has moved away from the epidermis and lies deep to the circular muscle layer. This difference appears to be associated with the position of the giant nerve

cells in the cord, of which there are one or two near the hinder limit of every segment. The cells generally occupy a mid-ventral position within the cord, but in *claparedii* they lie more laterally, beside rather than within the cord (Gamble & Ashworth, 1900 ; Nicol, 1948 ; Wells, 1958).

We have examined serial sections of well-fixed material of *pacifica* and *vagabunda* from False Bay, and find that the condition of the nerve-cord in *vagabunda* resembles that in *claparedii*, the nerve cord lying between the circular muscle layer and the epidermis and bearing its giant cells laterally. In *pacifica* the cord seems to be arrested in the act of crossing the circular layer, for some of the fibres run superficial and some deep to the cord ; the giant cells have moved towards the mid-ventral position (Plate 4).

Our sections of a few individuals from other localities are hardly suitable for neurological study, but they show the position of the nerve cord relative to the main layers. In two specimens of *oceanica* (from Dutch Harbour, Unalaska and Crescent City, California) the nerve cord lies close to the epidermis as in *vagabunda* and *claparedii*. The position in the circular muscle layer, shown by *pacifica* from False Bay, is seen also in specimens of the same form from Nanaimo, B.C., and Akkeshi, Japan.

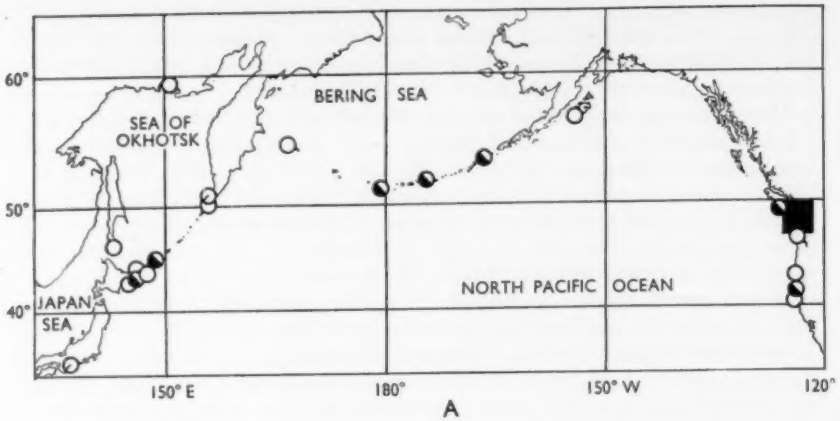
The anatomical comparison leads to the following conclusions. The four forms have certain common features which unite them into an apparently natural group and distinguish them from other Arenicolidae. Within the group, the least difference is that between *oceanica* and *vagabunda*, a matter only of the number of oesophageal caeca. The original neapolitan form approaches these two in its hooded nephridiopores and in the position of its nerve cord, and *pacifica* is perhaps the most distinct of the four.

GEOGRAPHICAL DISTRIBUTION

The only one of the four forms known to occur in European waters is *claparedii*. The specimens used by Levinsen in his original description of the species were from Naples where the same form is still to be found. It has since been collected at Santander. It is absent from the North Pacific area.

We shall consider separately the region incompletely cut off from the Pacific ocean by Vancouver Island, and including the Strait of Juan de Fuca, the San Juan Islands, the Strait of Georgia and Puget Sound (Fig. 1, lower part). E. A. H. has collected from over forty points in this region and several persons have been kind enough to send us material from others. The most commonly encountered lugworm in the region is *pacifica* which extends from Neah Bay at the mouth of the Strait of Juan de Fuca to near Tacoma in the south and at least as far as Kye Bay in the north. Apparently less widespread is *vagabunda*, which we have seen from seven localities only, all on or near the San Juan Islands. It is of course a possibility that this form, which is generally found near extreme low-water mark, may have a more extensive distribution beyond the tidal zone. Where they both occur in the same place, *pacifica* and *vagabunda* show ecological separation, as described in the next section.

The first lugworms to come from this region were recorded as *Arenicola claparedii* by Johnson (1901). His descriptions and illustrations show that


60°
50°
40°
SEA OF OKHOTSK
BERING SEA
NORTH PACIFIC OCEAN
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150° E
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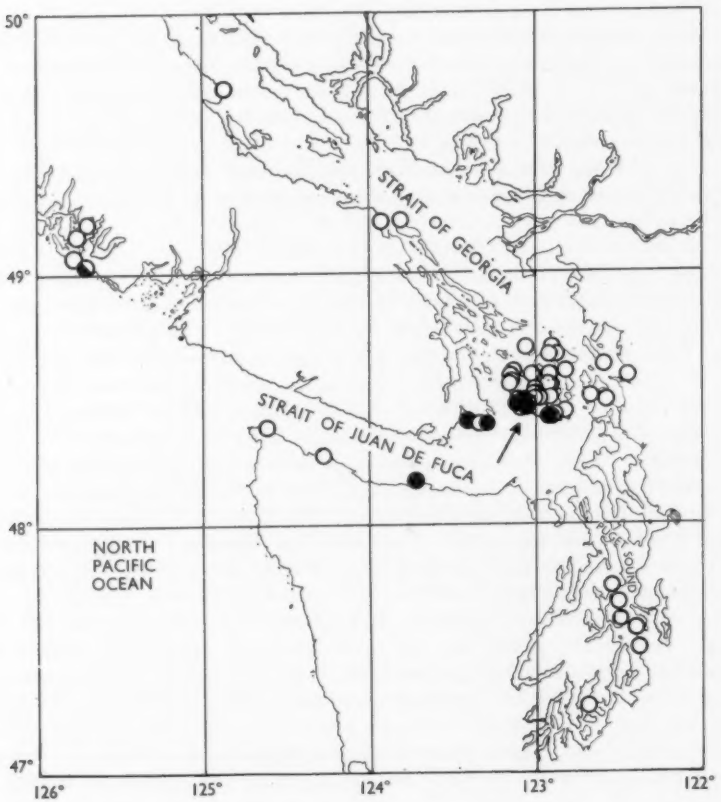
50°
49°
48°
47°
STRAIT OF GEORGIA
STRAIT OF JUAN DE FUCA
NORTH PACIFIC OCEAN
SOUND
126°
125°
124°
123°
122°

B

his material consisted of four specimens each of *pacifica* and *vagabunda*. They were collected by the Columbia University Zoological Expedition of 1896, and the exact localities were not stated. It seems very likely, from the general accounts of the expedition, that they came from the neighbourhood of Port Townsend (Dean *et al.*, 1897 ; Harrington & Griffin, 1897). This would slightly extend the distribution of *vagabunda*, as found by us, in the direction of Puget Sound.

Our information about the vast remainder of the North Pacific area is mainly derived from the examination by G. P. W. of the material listed in the Appendix (p. 332). He has been so fortunate as to secure the use of a private collection left at Edinburgh by the late Professor J. H. Ashworth, and has examined the relevant material in the British Museum of Natural History and the U.S. National Museum. Addition specimens were kindly sent by Mr and Mrs Berkeley, Dr Olga Hartmann and Professor P. V. Uschakov.

As shown in Fig. 1, *vagabunda* is restricted to the waters cut off by Vancouver Island ; outside this region, on the Pacific beaches, it is replaced by *oceanica*, which is morphologically very similar. Both *pacifica* and *oceanica* range from California through Alaska to Siberia and Japan, without obvious geographical separation. The fact that in nearly every case each locality has yielded only one of the two forms—the exceptions being Long Bay, Vancouver Island and Shikotan, Kurile Islands—suggests that there is an ecological separation of *pacifica* from *oceanica*, like that of *pacifica* from *vagabunda*.

BIOLOGY OF *PACIFICA* AND *VAGABUNDA*

To judge from preserved material alone whether the differences between the four forms are sufficient to justify distinction as species is a somewhat arbitrary procedure. Happily, *pacifica* and *vagabunda* are both abundant in False Bay, San Juan Island, Wash., U.S.A., which has a large intertidal area and is readily accessible from the Friday Harbor Laboratory of the University of Washington, so their ecology and behaviour can be compared. An air photograph of this important locality is reproduced in Plate 1, and its position is indicated by an arrow in the lower half of Fig. 1.

Distribution in False Bay

False Bay is shallow, roughly circular, and about two-thirds of a mile across. It opens to the south, with a mouth about one-third of a mile wide, between steep, rocky jaws. The whole bay, up to the mouth, empties on a favourable tide. Outside the mouth, the depth of the water increases rapidly. The flat, sandy floor of the bay slopes gradually towards the mouth and grades in texture from loose, deep sand at the mouth to a very fine, firmly packed material as the margins of the bay are approached. The floor is marked by

Fig. 1.—Geographical distribution of *pacifica* (white circles), *vagabunda* (black circles) and *oceanica* (black and white circles). The area of the lower map is shown as a black rectangle in the upper one. The arrow in the lower map indicates False Bay. All of the circles represent specimens seen by ourselves except for the two *pacifica* circles distinguished by oblique radii in the upper map (see Appendix, p. 332, for details).

broad, very shallow undulations running parallel to the entrance; these can be clearly seen in Plate 1. At low tide the flat crests of the undulations are drained and often ripple-marked, while the troughs are smooth, wet and sticky. Round the margin of the bay is a more noticeably sloping beach in which fine sand is mixed with gravel, stones and sometimes large boulders. During the summer months, much organic material, such as fragments of Ulva, is stranded on the marginal zone by the tides, and on a warm day can be detected some distance inland by the human nose. Two streams run into the bay, but carry little water during the summer months. The larger stream has produced a conspicuous " gravel fan " on the north side of the bay, opposite to the mouth (see Plate 1).

The distribution of the two forms, as shown in Fig. 1, is based on five daily visits made by G. P. W. from 10th to 14th August 1954. The bay was crossed along a number of straight lines, using landmarks on the shore, and the distribution of lugworms was noted from the castings. In as many places as possible, shown as circles on the map, worms were collected for subsequent identification in the laboratory.

One finds *vagabunda* in abundance in the bars of deep, loose sand at the mouth of the bay, a region seldom exposed except by the most favourable tides. Most of the worms on the sand bars are large, but a " nursery " of very small *vagabunda* was found on a sandbank just inside the east jaw of the mouth. Sparsely scattered individuals of this species occur on the flat floor of the bay, but they become steadily less frequent as one moves away from the mouth and the sand becomes finer and firmer. There is however a shallow drainage channel, running from below the gravel fan in the direction of the mouth, along which *vagabunda* penetrates further into the bay than elsewhere.

One finds *pacifica* round the gravelly and stony margins of the bay, where tidal exposure is frequent and the amount of stranded organic material is high. The great majority of the worms are much smaller than the *vagabunda* of the sand bars, but two patches of firm sand were found, just below the stony zone, one on the west side of the bay and one near the gravel fan, where large *pacifica* could be collected.

The zones inhabited by the two forms are completely distinct and separated, except along the drainage channel, by an extensive worm-free belt of very fine, firmly-packed sand. There was no overlap between the two populations. The single, large individual of *pacifica* found in the *vagabunda* zone, near the lower end of the drainage channel, and appearing as a white circle in Fig. 1, was presumably a stray, carried down the channel by the stream.

The above picture is by no means rigidly fixed, but varies in detail from year to year. The course of the main drainage channel was rather different in 1954 from that shown in the air photograph of 1949 (Plate 1). In 1958, comparatively few *vagabunda* were exposed, and those were confined to the outermost sand bars at the mouth of the bay ; on the other hand, *pacifica* had advanced some distance on to the floor of the bay from the western side since 1954.

In localities other than False Bay, *vagabunda* is restricted to deep, apparently mud-free sand. Sometimes *pacifica* occurs in sand resembling that favoured

by *vagabunda*, but this is rare ; more usually it is found in mud, gravel or various mixtures of mud and gravel with sand. The *vagabunda* sites are generally far down the beach and exposed only by very favourable tides, but an exception occurs in a small, unnamed bay just to the west of False Bay (between False and Kanaka Bays). Here a sand-bank with *vagabunda* lies at a higher level than a muddy bottom with *pacifica*, an observation which suggests that the distribution of *vagabunda* is limited by the nature of the substratum rather than duration of exposure, or other factors dependent on the tidal level.

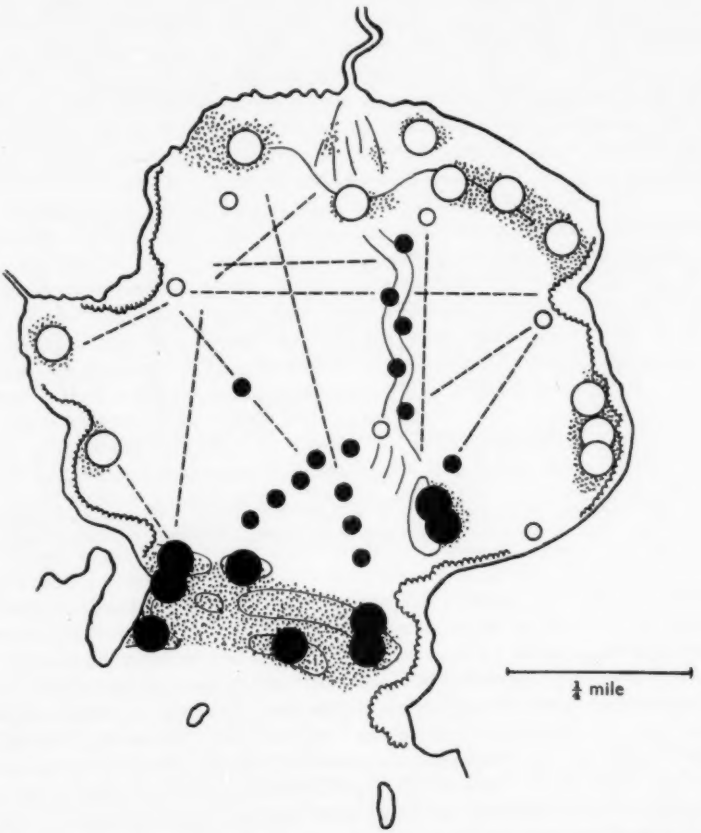

Fig. 2.—The distribution of *pacifica* (white circles) and *vagabunda* (black circles) in False Bay in August 1954. The circles are points from which worms were taken for identification. Dense lugworm populations are shown as dotted areas and large circles. Sparse or isolated individuals appear as small circles. The ruled lines represent walks along which no castings were seen. Compare Plate 1.

Forms of the burrows

The burrow of *Arenicola marina* was studied in detail by G. P. W. near Bangor, North Wales (Wells, 1945). At his suggestion, the class in Advanced Invertebrate Zoology at the Friday Harbor Laboratory, working under Professor Ralph I. Smith, made comparable observations at False Bay on 8th July 1953, and have kindly put their report at our disposal During the following weeks E. A. H., who had been a member of the class, collected further information in False Bay and made the sketches on which Fig. 3 is based.

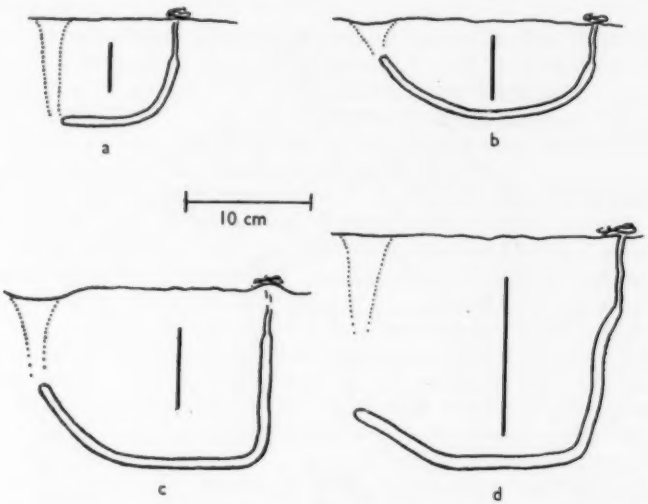

Fig. 3.—Four burrows of *pacifica*. Outlines of head shaft dotted. The vertical line down the centre of each drawing shows the length of the worm. All were in fine muddy sand except *a* which was in sand in the gravel fan.

The burrow of *A. marina* is a complicated structure, having as a whole a roughly U-shaped configuration, but comprising three well-differentiated regions (Wells, 1945). The worm moves to and fro in a " gallery " with firmed, mucus-impregnated walls ; this part of the burrow consists of a more-or-less vertical shaft curving below to become horizontal. At the upper end of the gallery is a narrow " tail shaft " which houses the worm's tail when it comes to the surface to defaecate, and just above this are the well-known castings. The third part of the burrow, the " head shaft ", rises from the lower end of the gallery to the surface, and varies greatly in structure with the type of substratum in which the burrow happens to be made. Typically it consists of a column of surface material, worked upon and often dragged downwards by the worm, and continually subsiding as the worm consumes it from the lower end. This subsidence results in a saucer-shaped or funnel-shaped depression of the sand surface at the upper end of the head shaft.

Many burrows of *pacifica* were dug out. They agreed in general form with those of *marina* (Fig. 3). The head-shaft was commonly a broad cone of soft, readily probed material, some of it (such as pieces of green *Ulva*) obviously dragged down from the surface. In some cases the upper end of the head shaft was marked by a shallow, saucer-like depression of the surface but often no surface signs of the head shaft could be seen. Sometimes, when no surface signs were visible, the head shaft was found to end above in a sub-surface cavitation, as that of *marina* may occasionally do (Wells, 1945, Fig. 2f). Some burrows were found which had apparently no head shafts ; these may have been newly formed.

The close resemblance in mode of life between *marina* and *pacifica* is remarkable in view of their many structural differences. One would expect some, at least, of these differences to be functionally significant ; for example, statocysts are present in *marina* but absent in *pacifica*.

The burrows of *vagabunda* are harder to study, as they are formed in deep, loose, water-filled sand. They give an impression of impermanence. Usually there is only a single casting above the tail shaft with no sign that the castings accumulate into heaps. Several vertical galleries were exposed, descending from the casting, some curving to become horizontal at a depth of ten inches or a foot, and others apparently ending at about that depth without any curvature. In spite of much searching, no definite head shafts were found, nor was there any depression of the sand surface marking the head workings of the worm below. Appearances suggest that this form, in colonising the loose, shifting sand, has abandoned the comparatively stable burrow for a life of continual improvisation. It is by no means an unsuccessful life. There may be as many as half-a-dozen castings per square foot, and the number of worms is considerably greater than the number of castings.

The sand in the *pacifica* zone often contains large amounts of wood chips, because the surrounding area was logged off about 1930 and the sand flats were used for tying up log booms. The chips are generally found in a compact layer under a foot or more of fine sand. It should not be inferred from this that the whole of the overlying sand has been deposited later than the chips. As lugworms (*A. marina*) feed from the lower ends of their head shafts, they reject such objects as shells or small stones which have come down the shafts. This may cause the formation of a definite layer of coarse material at the level of the bottoms of the head shafts, in beaches densely populated by *marina* (Wells, 1945, p. 186 ; van Straaten, 1952, 1956). As the mode of life of *pacifica* is very similar, the worms may have played an active part in burying the chips.

Commensal pea crabs

The following citation is from MacGinitie & MacGinitie (1949, p. 209) : " Even the lugworms . . . are sometimes hosts to pea crabs. In Puget Sound *Pinnixa eburnea* is commensal with *Arenicola claparedii*. Although we searched diligently for this crab, we found it with only those worms that were living in a sandy bar. The thousands of *Arenicola* in an adjacent mud flat did not harbor any commensal. "

This statement suggests the possibility that commensal pea crabs can distinguish between the different lugworms in the Puget Sound area. We asked Dr Dixy Lee Ray whether any evidence on this point could be found during the field work of the Advanced Invertebrate Zoology Class of 1958, of which she was in charge. She reported as follows on a visit to False Bay on 29th July. The class first dug in stiff muddy sand in the *pacifica* zone, a short distance inside the west jaw of the mouth. They found fourteen *pacifica*, about one quarter of the total number dug out, to have a commensal pea crab, and in every case the species was *P. schmitti*. This species also occurs with other mud-dwelling animals. The class then turned to the *vagabunda* area on the sand bars. Of about fifty *vagabunda* dug out six had commensal pea crabs, and in all cases these were *P. eburnea*. So far so good ; but on August 26th, E. A. H. dug out some large *pacifica* from sand much like that favoured by *vagabunda*, and found that three of these were in cohabitation, in each case with *P. eburnea*. It may therefore be that the substratum, rather than the worm, determines the crab.

Breeding season

Although our data on this point are regrettably incomplete, there are indications that *pacifica* and *vagabunda* may be prevented from interbreeding not only by their ecological separation but also by a difference in breeding season.

Many workers at the Friday Harbor laboratories have noticed that the body fluid of *vagabunda* is thick with ripe eggs and sperm clusters in June, July and August. At the same season, the body fluid of *pacifica* is only slightly turbid and such developing sex cells as it contains are few and obviously immature. E. A. H. has made successful fertilisations of *vagabunda* eggs, resulting in trochophore larvae, at various dates from 1st July to early August, but all of his attempts to fertilise the eggs of *pacifica* at the same season were unsuccessful. We have no information about the sexual condition of the False Bay worms in other months.

Dr Pierre Gonse studied the respiration of the developing oocytes of *pacifica* at Seattle in October, November and December 1957. His worms came, partly from Argyle Lagoon on San Juan Island and partly from localities near to Seattle. He has informed us that in October the worms contained only white, transparent, biconvex oocytes about 30 μ in diameter. By mid December all stages were found, from extremely immature oocytes like those just described to black, biconcave discs 190 μ in diameter. These may have been mature, but no fertilisations were tried ; the fact that many smaller stages were also present may be borne in mind.

Dr Gonse also examined the oocytes of a single batch of *vagabunda*, collected in mid November at Crescent Bay, on the south side of Juan de Fuca Strait. Small to medium-sized oocytes were found, the largest about 150 μ in diameter. The oocytes resembled in number and in stage of development those of *pacifica* at the same time, but those of vagabunda were thinner discs than those of *pacifica*, and therefore clear yellow instead of brown.

The facts are perhaps consistent with the view that *pacifica* breeds rather earlier in the year than *vagabunda* ; it would be most desirable to clear up the

question by means of a round-the-year watch on the sexual condition of both forms.

Spawning behaviour. "*Surfacing*" *of* vagabunda

Two authorities have published widely divergent accounts of the process of spawning in "*Arenicola claparedii*".

Guberlet (1934)* described observations made on "the sand flats of False Bay" and wrote :,

> "When the worms spawn they come to the surface and crawl along on top of the moist sand or burrow along in the surface only partly covered. They may be exposed to the sun in the middle of the day for three or four hours. The temperature of the sand is raised considerably during this period of exposure. Here they discharge their sexual products over the sand with the coelomic fluid. No distinct egg mass is formed. The spawned material of the female, filled with eggs, is of a watery consistency, and is red, orange, brown or grey in colour. The sperm material is somewhat lighter in colour but rarely white. These discharges seem to be made, when not disturbed, at about the time of the incoming tide so that the circulation of the water brings the sperm and eggs together. The eggs, immediately after fertilisation, become adhesive and adhere to the sand".

Guberlet witnessed spawning in 1932 on July 18th, 19th, 20th and 21st ; "at the end of this period practically no sex cells could be found in the coelomic fluid of the worms". In the previous year, emergence on the surface occurred in late June and early July ; by 13th July the worms were "spawned out" and "only a few worms were on the surface of the sand".

The account of Okuda (1946, pp. 162–3) is based on worms kept in an aquarium at the Akkeshi Marine Biological Station in Japan. The worms breed from the latter part of April to the first of June. There is no emergence of the worms on the surface. Okuda describes the process of spawning as follows :

> "First the male discharged the spermatophore through his nephridiopores by a peristaltic movement of the body. The discharged spermatophore was an oval pellet, 0·5–2 mm. in diameter, and enveloped with a thin gelatinous membrane. . . The spermatophores were shot out one by one, ten to twenty per minute, into water from the burrow of the male worm. The process reminded one of an intermittent eruption of a minute volcano. The ejection of the spermatophores continued for about two hours ; the number ejected decreased gradually as time went on. The discharged pellets were deposited around the entrance of the burrow, and some of them were observed to flow accidentally into an adjacent hole in which a female lives. . . . When a spermatophore sank into the burrow of a female worm which lay adjacent to the male, it was caught by the bristles. The membrane of the spermatophore was so thin as to easily burst and the spermatozoa thus liberated swam about actively in water. At this time oviposition began. The unfertilised eggs, slightly yellow in colour, were discharged first from the last nephridiopore, then from the fourth, third, second and

* Guberlet follows Ashworth in merging *claparedii* with *pusilla*.

finally from the first. This process was repeated several times in accordance with a peristaltic movement of the body. The eggs which were fertilised outside the body gradually settled down into a gap of the burrow occupied by the female worm. By the time when the spawning which had lasted two or three hours was over, the eggs had accumulated outside the body into a cylindrical tube."

Any suspicion that the process just described is abnormal because of the confinement of the worms in an aquarium is stilled by the fact that a similar process has been seen to occur in shallow pools on the beach at Akkeshi : " I saw here and there spermatophore pellets being actively ejected from a male hole in the manner described above " (Okuda, 1938).

It will be noted that there is hardly any point on which the two authorities agree, and the explanation for this wide discrepancy may be that they were working on different forms. There is little doubt that Guberlet's description applies to *vagabunda*, which lives on the sand flats and matures in late summer. Some specimens of "*A. claparedii*" from Akkeshi were sent by Dr Okuda to the late Professor J. H. Ashworth. These specimens are now in the possession of G. P. W. and they agree in every respect with *pacifica*.

As far as we are aware, no observations have been made on the spawning of *pacifica* in False Bay or the surrounding region.

The "surfacing" of *vagabunda*, in the manner described by Guberlet, was witnessed on the following occasions during the course of our work ; by the Invertebrate Zoology Class on 8th July 1953, when many worms emerged ; by E. A. H. a month later, when only a few appeared ; by G. P. W. on the following dates in 1954 : June 20th and 29th, July 30th, August 10th and 12th.

We find that surfacing generally occurs as the tide begins to return over the sand bars. Usually the worm is first seen at a casting, or near a casting and moving away from it, as if the surface had been reached by climbing up the tail shaft, and several worms were dug out as they were doing just that. Sometimes it crawls actively over the surface, sometimes parallel with the surface but a little below ; in the latter case the thin " roof " of sand over the worm may crack and expose its back. The name *vagabunda* was suggested by these conspicuous wanderings.

The great majority of the worms crawling on the surface were large and full of ripe eggs or sperms, but occasionally immature worms were found wandering. The surfacing worms of 12th August 1954 included two small individuals, measuring respectively 34 and 54 mm. from mouth to tail base, whose coelomic fluid was clear and therefore free from developing sex cells. It appears from Guberlet's account that *vagabunda* spawns while crawling on the surface ; on the other hand, it need not be true that the worms appear at the surface only when about to spawn. As far as we are aware, nobody has collected from the sand bars during the winter months when *vagabunda* is presumably unprepared for breeding. It would be interesting to know whether they surface at that season. The burrows of *vagabunda* have an impermanent appearance, as described in an earlier section, so it may be that this form is a frequent and persistent wanderer.

DISCUSSION

If attention be concentrated on the situation in False Bay, the case for describing *pacifica* and *vagabunda* as separate species is seen to be strong. They differ in several anatomical characters and can hardly be confused with each other even if they have been very badly preserved. They are ecologically distinct, and probably differ in breeding season and breeding habits. No intermediates—that is, individuals which cannot be referred without hesitation to one form or the other—have been found throughout the region where they co-exist.

There is no reason—supposing that *pacifica* and *vagabunda* are separate species—for identifying either with *claparedii*. The European form resembles *pacifica* in the number of its oesophageal caeca but resembles *vagabunda* in its hooded nephridiopores and superficially-placed nerve cord, and is prevented by the inflexible facts of geography from interbreeding with either. It seems therefore desirable to describe *pacifica* and *vagabunda* as new species, distinct from *claparedii* Levinsen. On the other hand *oceanica* is best regarded as a subspecies of *vagabunda*, which it replaces geographically and from which it differs only in the number of oesophageal caeca.

Hitherto the forms here separated as *claparedii*, *pacifica*, *vagabunda* and *oceanica* have always been referred to a single species, and it should not be forgotten that their close relationship is shown by certain common characters ; the combination of the absence of statocysts with the great muscular development of the more anterior chaetigerous annuli and concomitant reduction of the intervening ordinary annuli distinguishes them from other lugworms. They have five pairs of nephridia. The remaining species of *Abarenicola* are *assimilis* (Ehlers), which has statocysts and (generally) six pairs of nephridia, and *pusilla* (Quatrefages) which lacks statocysts but has six pairs of nephridia, lacks the powerful development of the more anterior chaetigerous annuli, and shows a unique feature of the neuropodia, those of setigers vi and vii being the shortest (Wells, 1954).

DEFINITIONS

(*Abarenicola* Wells is distinguished from *Arenicola* Lamarck by the following characters : Prostomium non-retractile, in the form of a triangle with lateral extensions of its (anterior) base ; none of the neuropodia approaches close to the mid-ventral line ; oesophageal caeca more than one pair ; septal pouches absent. It includes *assimilis* (Ehlers, 1897) and *pusilla* (Quatrefages, 1865) in addition to the forms described below.)

Abarenicola claparedii (Levinsen, 1883).

Abarenicola with 19 setigers. Gills 13 pairs, on setigers vii to xix. Nephridia five pairs opening on setigers v to ix. Statocysts absent. The chaetigerous annuli of the first three or four trunk segments are very powerfully developed, and the intervening ordinary annuli are correspondingly reduced. Nephridiopores hooded. Oesophageal caeca 1 + 3 or 1 + 4.

Europe (Mediterranean, Santander).

No type specimens were designated. The species was based on material from Naples, where it is still to be found, and the original specimens doubtless resembled the present-day Neapolitan ones.

Abarenicola pacifica. Sp. nov.

Abarenicola with 19 setigers. Gills 13 pairs, on setigers vii to xix. Nephridia five pairs opening on setigers v to ix. Statocysts absent. The chaetigerous annuli of the first three or four trunk segments are very powerfully developed and the intervening ordinary annuli are correspondingly reduced. Nephridiopores naked. Oesophageal caeca 1 + 3 to 1 + 6.

North Pacific (California through Alaska to Japan ; San Juan Islands and surrounding region).

Holotype from False Bay, desposited in the British Museum (Natural History), Catalogue No. 1958 : 9 : 2 : 1. Paratypes from the same batch of material in the U.S. National Museum, Washington D.C., and the Washington State Museum, Seattle. Photograph of holotype in Plate 1 of this paper.

Abarenicola vagabunda. Sp. nov.

Abarenicola with 19 setigers. Gills 13 pairs, on setigers vii to xix. Nephridia five pairs opening on setigers v to ix. Statocysts absent. The chaetigerous annuli of the first three or four trunk segments are very powerfully developed and the intervening ordinary annuli are correspondingly reduced. Nephridiopores hooded. Oesophageal caeca 1 + 7 or more.

The specific name refers to the habit of wandering on the surface of the sand.

A. v. vagabunda.

Oesophageal caeca 1 + 11 to 1 + 18.

Apparently restricted to the San Juan Islands and surrounding region.

Holotype from False Bay, deposited in the British Museum (Natural History), Catalogue No. 1958 : 9 : 2 : 2. Paratypes from the same batch of material in the U.S. National Museum, Washington, D.C., and the Washington State Museum, Seattle. Photograph of holotype in Plate 1 of this paper.

A. v. oceanica subsp. nov.

Oesophageal caeca 1 + 7 to 1 + 9.

North Pacific (California through Alaska to Japan, but absent from the San Juan Islands and surrounding region, where it is replaced by *A. v. vagabunda*).

Holotype from Dutch Harbour, Unalaska, deposited in the British Museum (Natural History), catalogue No. 1958 : 9 : 2 : 3. Paratypes in the U.S.A. National Museum, Washington, D.C., and in the Washington State Museum, Seattle. The types are taken from a batch of material in the Ashworth collection, labelled " Dutch Harbour, Unalaska. August 1910 ".

ACKNOWLEDGMENTS

We have pleasure in thanking Dr Dixy L. Ray of the University of Washington for moral support and physical assistance—the former including the criticism of our typescript and the latter much digging and the frequent loan to G. P. W. of her long-suffering automobile.

We are grateful to the many colleagues who have sent us material, and especially to Mr and Mrs Berkeley, of the Biological Station, Nanaimo, who in addition have read and commented on our typescript.

SUMMARY

1. Worms currently referred to *Abarenicola claparedii* Levinsen are found in Europe and over a wide range in the North Pacific from Japan through Alaska to California. On the basis of a series of specimens from localities covering the entire range, it is shown that the North Pacific worms belong to three forms differing in anatomy from each other and from the original European one. The Pacific forms are described as two new species (*A. pacifica* and *A. vagabunda*) and a distinct subspecies of the latter (*A. v. oceanica*). The name *claparedii* is restricted to the European form. The characters by which the forms are diagnosed (number of oesophageal caeca, presence or absence of a hood over the nephridiopore) have not hitherto been regarded as of taxonomic importance in the Arenicolidae.

2. The biology of *pacifica* and *vagabunda* was studied at False Bay, San Juan Island, Wash., U.S.A., where they occur side by side. They live in different parts of the bay and characteristically in different substrata—*vagabunda* being confined to deep sand near the mouth of the bay and *pacifica* being generally found in gravelly or muddy material round its margin. They probably differ in breeding season and in breeding behaviour. The burrows of *pacifica* are very similar to those of *Arenicola marina*; those of *vagabunda* appear to be less permanent. The commensal crabs *Pinnixa eburnea* and and *P. schmitti* are commonly found with *vagabunda* and *pacifica* respectively, but it may be the substratum, rather than the worm, that determines the crab.

APPENDIX

List of specimens from Pacific localities other than the region of the San Juan Islands, Puget Sound, Strait of Georgia and Strait of Juan de Fuca. The following localities are shown as circles in the upper half of Fig. 1 (p. 320), except O.2 and P. 4, 5, 6, which appear in the lower half.

OCEANICA

O. 1 Crescent City, California. Ashworth collection. One worm, labelled " Harvard coll. 91 ". The prostomium of this specimen is figured in Ashworth 1912, Figs. 53, 54. It is one of the four recorded by Gamble & Ashworth (1900) as the first *A. claparedii* from the Pacific.

O. 2 Long Bay, just S. of Tofino, Vancouver Island.	From E. and C. Berkeley. One worm. Collected August 1931.
O. 3 Sunday Rock, Estevan Point, Vancouver Island.	From E. and C. Berkeley. One worm. Collected by E. G. Hart in 1934. Recorded by Berkeley & Berkeley (1942).
O. 4 Dutch Harbour, Unalaska.	(a) From Olga Hartman. One worm. Coll. W. R. Coe, 10th July 1899. Harriman Alaska Expedition. (b) Ashworth collection. Two worms. Coll. 10th July 1899. Harriman Expedition. (c) Ashworth collection. Six worms. Dated " August 1910 ". Here designated as holotype and paratypes of *oceanica*. (d) British Museum (Nat. Hist.) 1912 : 4 : 9 : 28. One worm. Coll. H. Heath, August 1910. Presumably from the same batch as the types.
O. 5 Atka Island, Alaska.	(a) U.S. National Museum 16408. Three worms. Coll. 10th June, 1894. Steamer Albatross (U.S. Fish Commission). Label adds " one in exchange to J. H. Ashworth, 12th June, 1912 ". (b) Ashworth collection. One worm. Presumably the one mentioned in (a). Prostomium figured in Ashworth 1912, Fig. 49.
O. 6 Constantine Harbour, Amchitka Island, Alaska.	U.S. National Museum 16410. One worm. Coll. W. H. Dall, 5th August, 1873.
O. 7 Iturup, Kurile Islands.	From P. V. Uschakov. Three worms. Coll. O. Kussakin, 1954.
O. 8 Shikotan, Kurile Islands.	Zool. Inst., Acad. Sci., Leningrad, No. 18/6959. Two worms. Coll. Gurjanova, 1949.
	PACIFICA
P. 1 Sand Point, Humbolt Bay, California.	U.S. National Museum 16409. One worm. Steamer Albatross.
P. 2 Coos Bay, Oregon.	From Olga Hartman. One worm. Coll. 1942, eel-grass mud, intertidal.
P. 3 Hoquiam, Wash.	Coll. E. A. H. Twelve worms. In very soft mud and gravel, May 1956.
P. 4 Long Bay, just S. of of Tofino, Vancouver Island.	From E. and C. Berkeley. One worm. Coll. August 1931.
P. 5 Tofino Inlet, Vancouver Island.	Ashworth collection. One worm.

P. 6 Head of Tofino Inlet, Vancouver Island.	From E. and C. Berkeley. Two worms. Coll. 1925, in very soft mud.
P. 7 Lazy Bay, Kodiak Island, Alaska.	(a) U.S. National Museum 23126. Twenty-eight worms. Coll. 12th Nov. 1940, in sand and gravel at low tide. Alaska King Crab Investigation, U.S.B.F. (b) U.S. National Museum 23125. One worm. Coll. 22nd Jan. 1941, in gravelly sand at head of bay. Alaska King Crab Investigation, U.S.B.F. Both were recorded by Hartman (1948).
P. 8 Bering Island.	Zool. Inst., Acad. Sci., Leningrad, No. 15. One worm. Coll. Schmidt, 1929.
P. 9 Nagaeva Bay, Tauisk, Sea of Okhotsk.	Zool. Inst., Acad. Sci., Leningrad, No. 3. Two worms. Coll. Uschakov, 1949.
P. 10. Paramuschir, Kurile Islands.	Zool. Inst., Acad. Sci., Leningrad, No. 20/6961. Two worms. Coll. Korotkevich, 1954–55.
P. 11 Shikotan Kurile Islands.	Zool. Inst., Acad. Sci., Leningrad. Three worms. Coll. Kussakin, 1954–55.
P. 12 Kunashir, Kurile Islands.	Zool. Inst., Acad. Sci., Leningrad. No. 12. Five worms. Coll. Kussakin, 1952.
P. 13 Bussé Bay, Aniva, South Sakhalin.	Zool. Inst., Acad. Sci., Leningrad. No. 11. Three worms. Coll. Rosenthal, 1947.
P. 14 Akkeshi, Hokkaido, Japan.	Ashworth collection. Six worms. Sent to Ashworth by Shiro Okuda, 1932.

The records of *pacifica* from Alaid, Kurile Islands and Momotori, Japan (distinguished by oblique radii in Fig. 1) are based on the very clear descriptions and drawings of Okuda, 1933.

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EXPLANATION OF THE PLATES

PLATE 1

(1) Air photograph of False Bay, 4th June 1949. Reproduced by courtesy of U.S. Coast and Geodetic Survey.

(2) Side views, from the right, of the holotypes of *pacifica* (above) and *vagabunda* (below). The worms were collected from False Bay in August 1954 and narcotised in isotonic $MgCl_2$ before killing.

PLATE 2

Dissections from the dorsal surface of *pacifica* (3), *vagabunda* (4) and *claparedii* (5). The first two are from False Bay, the third from Naples. The caeca of these individuals number : *pacifica* 1 + 4 ; *vagabunda* 1 + 14/15 ; *claparedii* 1 + 3. The tips of the first (long) pair of caeca in *claparedii* have curled out of sight under the gut.

PLATE 3

On the left, three photographs, to the same scale, each showing one of the nephridiopores of the left side, marked by an indicator line, together with the adjacent notopodium and neuropodium. The animals are *pacifica* (6), *vagabunda* (7), and an artificially stretched *vagabunda* (8). In the last, a glimpse of the inner nephridiopore can be seen through the gaping opening of the hood.

On the right, four photographs, to the same scale, of serial transverse sections through the body wall, coelomic face to the left. The sections pass through a nephridiopore of *pacifica* (9), 60 μ anterior to the same nephridiopore (10), through a true (inner) nephridiopore of *vagabunda* (11) and 90 μ anterior to the same (12). Indicator lines show the nephridiopores. The nature of the hood in *vagabunda* is clearly shown.

All of the photographs on Plate 3 are of False Bay material.

PLATE 4

Photographs of a transverse section of the nerve cord and neighbouring tissues in *vagabunda* (13) and of sagittal sections, passing through the neuclei of giant cells, in *vagabunda*. (14) and *pacifica* (15). In each case an indicator line, of length corresponding to 0·2 mm., is drawn to the margin of a giant cell. The worms are from False Bay. Notice that, to show the giant cells, the sagittal section must be cut through the fibrous part of the cord in *pacifica* but to one side of it in *vagabunda*.

All photographs in the plates, except the air photograph in Plate 1, are by Mr W. Brackenbury.

NOTICE

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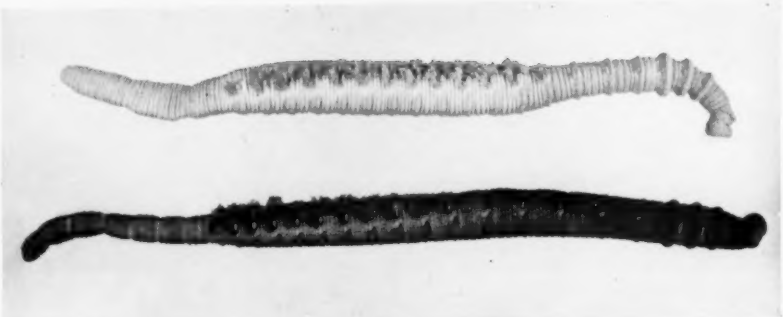
1. False Bay



2. *A. pacifica.*
A. vagabunda.

3. *A. pacifica.*

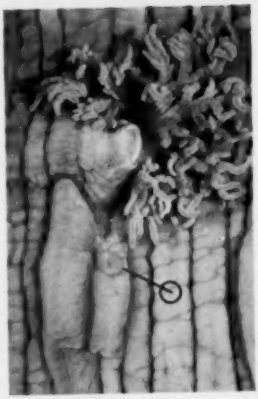
1 cm.

4. *A. vagabunda.*

1 cm.

5. *A. claparedii.*

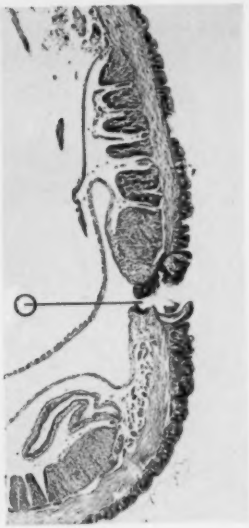
1 cm.



6

A. pacifica.

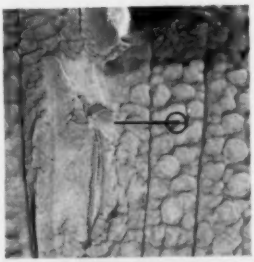
5 mm.



9

A. pacifica.

10

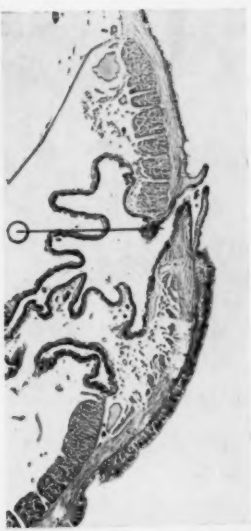


7

8

A. vagabunda.

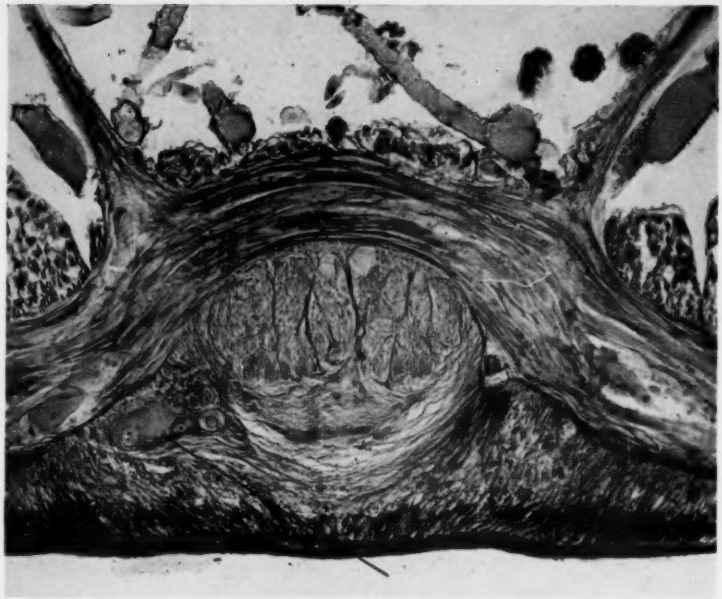
1 mm.



11

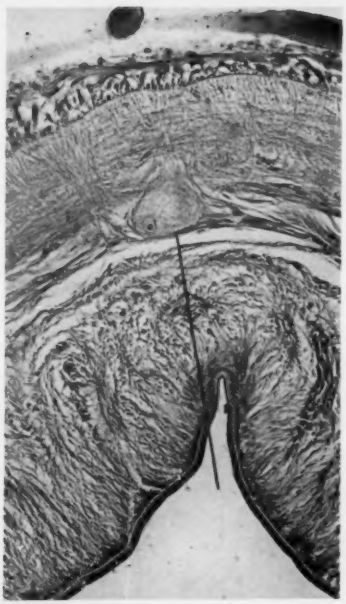
A. vagabunda.

12



13. *A. vagabunda.*

14. *A. vagabunda.*



15. *A. pacifica.*

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LIST OF CONTENTS

VOL. 133

PART 2 (pp. 171–335)

Page

CRANBROOK, The Earl of
The feeding habits of the water shrew *Neomys fodiens bicolor* Shaw, in captivity and the effect of its attack upon its prey 245

HARRISON, J. L.
Defaecation in the flying lemur *Cynocephalus variegatus*. (With 1 plate) 179

HEALY, Eugene A. and WELLS, G. P.
Three new lugworms (Arenicolidae, Polychaeta) from the North Pacific area. (4 plates and 3 figures) 315

POPHAM, Edward J.
The anatomy in relation to feeding habits of *Forficula auricularia* L. and other Dermaptera. (28 figures) 251

RALPH, Patricia M.
The status and affinities of the anthomedusan *Paragotoea bathybia* Kramp, 1942. (1 figure) 171

SIMKISS, K. See TYLER, C.

TYLER, C. and SIMKISS, K.
A study of the egg shells of ratite birds. (3 plates and 12 figures) .. 201

WELLS, G. P.
The genera of Arenicolidae (Polychaeta). (2 plates and 2 figures) .. 301

WELLS, G. P. See HEALY, Eugene, A.

WOODHEAD, A. D. See WOODHEAD, P. M. J.

WOODHEAD, P. M. J. and WOODHEAD, A. D.
The effects of low temperatures on the physiology and distribution of the cod, *Gadus morhua* L., in the Barents Sea. (1 plate and 4 figures) 181

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